VOLUME VII JULY, 1924 NUMBER 4

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

Contents

Intrauterine Development of the Bovine Fetus in Relation to Milk Yield in Guernsey Cattle—JOHN W. GOWEN 311
IV. Sodium Hypochlorite. The Phenol Coefficient and Relative Disinfecting Power of Sodium Hypochlorite—HARPER F. ZOLLER AND SYLVIA M. EATON 318
The Bacterial Content of Ice Cream—A. C. FAY AND N. E. OLSON 330
The Relation Between Yeasts and Moulds and the Keeping Quality of Butter—DONALD BETHUNE SHUTT 357
Adulterated Acid as a Possible Source of Error in Testing Milk by the Babcock Method—WM. E. PETERSEN 361
The Influence of Pasteurization and Diet of the Cow on the Anti-Scorbutic Potency of the Milk—THOMAS M. OLSON AND LYMAN COPELAND 370
A Colorimetric Picric Acid Method for Determining Lactose—H. B. BIERMAN AND F. J. DOAN 381
The Official Test in the South and the Preliminary Milking—EARLE BRINTNALL 393
Summary of Minutes of Meeting of Southern Division, American Dairy Science Association, Birmingham, Alabama, January 10, 1924—J. A. GAMBLE 403
Review of Foreign Dairy Literature—H. A. BENDIXEN 407
Dairy Notes 411
American Dairy Science Association Members for 1924 413

PUBLISHED BI-MONTHLY
WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.
Copyright, 1924, by Williams & Wilkins Company
Made in United States of America

The Cherry Twin Coil in Pasteurization

In addition to the fact that the Cherry Milk Pasteurizer is the best built, best insulated and most scientifically engineered machine of its kind ever built, it has the added advantage of being equipped with the

Cherry Twin Coil

Two independent streams of the heating or cooling liquid pass through this coil. Each stream goes through a separate coil, giving practically double the flow found in other types of coils. This means there is no "cold" end of the coil and that the batch temperature is uniform **throughout.**

Do not confuse the Cherry patented Twin Coil with ordinary "double" coils or with coils in which the liquid passes from end to end and back again through alternate coils.

What This Coil Does

It speeds up output by bringing batch to desired temperature in less time.
It brings ALL the batch to the same temperature.
It permits holding temperatures more positively.
Where cooling is done in the pasteurizer it enables the operator to do the work faster than is possible with any other coil.
It gives a larger output from the same size vat in which a single coil or infusion coil is used.

Send for Bulletin No. 231 giving specifications on all Cherry Coil Machines.

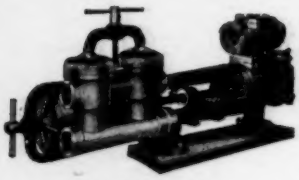
J.G. CHERRY COMPANY

ST. PAUL, MINN. — TAMA, IOWA — PEORIA, ILL.

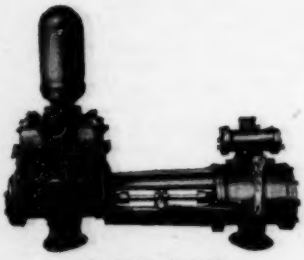
CEDAR RAPIDS IOWA

"DAVIDSON" PUMPS

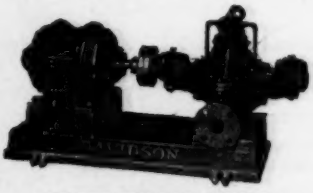
for Creameries and Milk Plants



MILK PUMP



WATER PUMP



CENTRIFUGAL PUMP

MILK PUMPS

BOILER-FEED PUMPS

BRINE-CIRCULATING PUMPS

TANK SUPPLY PUMPS

WASHER PUMPS

ARTESIAN WELL PUMPS

Strict adherence for more than 40 years to our policy of high grade materials and first-class workmanship have produced "The Best Pump Built"

Send for Catalog and References in Your Vicinity

M. T. DAVIDSON COMPANY

154 Nassau Street, New York

135 Oliver St., Boston, Mass.
1011 Chestnut St., Philadelphia, Pa.
1224 Granite Building, Rochester, N. Y.
604 Empire Building, Detroit, Mich.
817 Albee Building, Washington, D. C.
37 W. Van Buren Street, Chicago, Ill.

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL
OF
DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

J. H. FRANDSEN, Editor
Lincoln, Nebraska

ASSOCIATE EDITORS

F. W. BOUSKA, Chicago, Ill.
A. A. BORLAND, State College, Pa.
C. H. ECKLES, St. Paul, Minn.
R. C. FISHER, Storrs, Conn.
J. B. FITCH, Manhattan, Kans.
J. A. GAMBLE, College Park, Md.
E. S. GUTHRIE, Ithaca, N. Y.
H. A. HARDING, Detroit, Mich.
S. H. HARVEY, College Park, Md.
O. F. HUNZIKER, Chicago, Ill.
H. H. KILDEE, Ames, Iowa
C. W. LARSON, Washington, D. C.
H. F. JUDKINS, Amherst, Mass.
M. MORTENSEN, Ames, Iowa
LEROY S. PALMER, St. Paul, Minn.
T. R. PIRTLE, Washington, D. C.
C. L. ROADHOUSE, Davis, Calif.
L. A. ROGERS, Washington, D. C.
H. A. RUEHE, Urbana, Ill.
W. A. STOCKING, JR., Ithaca, N. Y.
H. E. VAN NORMAN, Washington, D. C.
IVAN C. WELD, Washington, D. C.
G. C. WHITE, Storrs, Conn.

OFFICERS OF AMERICAN DAIRY SCIENCE ASSOCIATION

President ..... A. A. BORLAND, State College, Pennsylvania
Vice President ..... O. E. REED, East Lansing, Michigan
Secretary-Treasurer ..... J. B. FITCH, Manhattan, Kansas

SECTION NO. 1—DAIRY PRODUCTION

Chairman ..... J. J. HOOPER, Lexington, Ky.
Secretary ..... J. A. GAMBLE, College Park, Md.

SECTION NO. 2—DAIRY MANUFACTURES

Chairman ..... H. W. GREGORY, Lafayette, Ind.
Secretary ..... R. C. FISHER, Storrs, Conn.

SECTION NO. 3—EXTENSION

Chairman ..... L. W. MORLEY, State College, Pa.
Secretary ..... C. A. HUTTON, Knoxville, Tenn.

SECTION NO. 4—OFFICIAL TESTING

Chairman ..... R. T. HARRIS, Madison, Wis.
Secretary ..... G. C. WHITE, Storrs, Conn.

Your advertisement is being read in every State and in 25 Foreign Countries

THE THOUSANDS WHO USE

NAFIS
Scientific Glassware

Year after Year, do so because they have found that it pays them to use it

They have found by experience that:—

Its ACCURACY is absolutely GUARANTEED and dependable.

The articles are made of a HIGH QUALITY of GLASS PROPERLY ANNEALED.

The PRICES are CONSISTENT with the HIGH VALUES GIVEN.

If your dealer does not stock NAFIS GLASSWARE write for our catalog and the names of our distributors in your territory.

LOUIS F. NAFIS, Inc.

Manufacturers of Scientific Glassware for Testing Milk and its Products

23 North Desplaines St. **CHICAGO, ILLS.**

ASK THE MEN WHO USE IT

Your advertisement is being read in every State and in 25 Foreign Countries

Candor in Advertising, Or, A Plain Confession

"How do they advertise," in asking about a business concern is in all respects comparable to, "How does he talk," when asked concerning an individual.

The favorite pastime of many ad-copy writers comes in the hunt for new superlatives. You have read advertisements, which, if said by word of mouth would forever stamp the speaker as a confirmed "Lightnin' Bill Jones."

We believe that the greatest virtue of advertising is candor.

Take, for example, our page and half page ads in the dairy trade journals. In those we illustrate the uses of B-K with simple unretouched photographs of actual scenes in creameries and cheese factories right here in Wisconsin.

The experiences with B-K of well known dairy factories are cited. Every word of our own text is backed by laboratory experiments and tests.

By simply illustrating the application of B-K and citing the recommendations of others, the expedient of "fireworks" in creating impressions, is happily avoided.

And we believe with most people that such candor in advertising implies honesty in manufacturing and sales policy.

We invite correspondence and criticisms.

GENERAL LABORATORIES

Madison, Wisconsin

Your advertisement is being read in every State and in 25 Foreign Countries

INTRAUTERINE DEVELOPMENT OF THE BOVINE FETUS IN RELATION TO MILK YIELD IN GUERNSEY CATTLE[1]

JOHN W. GOWEN

Maine Agricultural Experiment Station, Orono, Maine

Received for publication April, 1924

Experienced physicians and others who come in contact with obstetrical work in man or animals frequently hold the opinion that the development of the fetus is a serious drain on the animal organism. If this animal happens to be in milk production this supposed drain is said to produce a marked depression in the animal's milk yield. In view of the wide credence placed in this hypothesis it is surprising to find that the experimental evidence presented in its support is so slight. The American Guernsey Cattle Club in volume 31 of their advanced registry furnished data on the milk yields and length of time the calf is carried by the cows during the 365-day lactation in which the cows are making their record. These data are analyzed in the following paper to determine the validity of the hypothesis indicated above.

CORRELATION BETWEEN THE MILK YIELD AND DURATION OF PREGNANCY AND BETWEEN THE BUTTER-FAT PERCENTAGE AND DURATION OF PREGNANCY

The data are divided into periods of six months according to the age of the cow thus eliminating any marked influence of the cow's age on her milk yield. Cows on advanced registry tests are generally bred in or after the fourth month of lactation, the

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 156. The Rockefeller Institute for Medical Research has furnished material aid to these animal husbandry investigations in the form of a grant for increased maintenance of the work. This paper presents one phase of these researches as conducted by the Biological Laboratory of the Maine Agricultural Experiment Station.

idea being to have them calve after the 365-day test is finished. The duration of time the calf is carried during the test period varies from zero days to nine months. The cow's records for each six months of age have been sorted for 365-day milk yield and the duration of time the calf was carried. The milk yields range from 4000 to 24,000 pounds and are divided into classes of a thousand pounds. The duration of time the calf is carried varies from zero months to nine months and is divided into monthly classes. The correlation coefficients for the two variables, milk yield and butter-fat percentage with the duration of pregnancy are given in table 1.

The correlation coefficients of table 1 to some degree bear out the general impression that the fetus exerts a physiological drain on the mother and that this drain is expressed in a reduction in milk yield. The percentage composition of the milk solids as indicated by the butter-fat is not influenced by the presence of the fetus.

The influence of the length of time the calf is carried is least for the young and old cows and greatest as the cows reach the five-year old mark. There are other extraneous complicating factors entering into this relation. From the manner of conducting the advanced registry test it seems entirely probable that the owner will favor in every way possible the cow that he thinks is going to make a large record. Two things will influence his decision; the previous yearly records of the cow in question and the monthly record of the cow commencing this test. If this monthly record is high it would naturally follow that the dairyman believing in the influence of carrying the calf in tending to reduce the milk yield, would not breed his cow with probable high production so early as he would breed the cows which were going to make a relatively lower record. Study of the records show that such a practice is actually followed. The first four months of lactation are studied for the ages three years to three years six months and three years six months to four years. These records are chosen as they closely represent the average in the relation of the intrauterine developments of the fetus to milk yield. As the milk yields are recorded in calendar months, the

first month's record is anything from one to thirty-one days in length. As such a record is worth very little for our problem it is omitted. The correlation coefficients for the relation of the

TABLE 1

Correlation coefficients for milk yield with duration of pregnancy within the 365-day lactation (column 2) and butter-fat percentage with duration of pregnancy within the 365-day lactation (column 3)

AGE GROUPS OF COWS	MILK YIELD	BUTTER-FAT PERCENTAGE
1:6–2:0	−0.021 ± 0.059	−0.011 ± 0.059
2:0–2:6	−0.127 ± 0.018	−0.013 ± 0.018
2:6–3:0	−0.199 ± 0.024	0.089 ± 0.024
3:0–3:6	−0.175 ± 0.027	0.040 ± 0.028
3:6–4:0	−0.235 ± 0.029	0.015 ± 0.031
4:0–5:0	−0.275 ± 0.022	−0.022 ± 0.024
5:0–6:0	−0.228 ± 0.039	0.071 ± 0.030
6:0–8:0	−0.174 ± 0.024	−0.040 ± 0.025
8:0–10:0	−0.180 ± 0.036	−0.029 ± 0.037
10:0–15:6	−0.111 ± 0.057	0.041 ± 0.058
Average	−0.173	0.014

TABLE 2

Correlation coefficients for the monthly milk yield with time of interuterine development (column 2) and monthly milk yield with yearly milk yield (column 3)

MONTH OF LACTATION	CORRELATION COEFFICIENT	
	Monthly milk yield and duration of interuterine development	Monthly milk yield and 365-day milk yield
Three years to three years six months		
Second	−0.103 ± 0.028	0.757 ± 0.012
Third	−0.136 ± 0.028	0.807 ± 0.010
Fourth	−0.146 ± 0.028	0.834 ± 0.009
Three years six months to four years		
Second	−0.152 ± 0.031	0.713 ± 0.016
Third	−0.170 ± 0.030	0.784 ± 0.012
Fourth	−0.187 ± 0.030	0.812 ± 0.011

monthly milk yields and the duration of time the calf was carried in the 365-day lactation period and the correlation coefficients for the monthly milk yields and 365-day milk yields are given in table 2.

The second column of table 2, shows that the cows selected by the dairymen to carry the calf nine months are slightly less in their milk yield than are the cows selected to carry the calf one month or not at all. This fact is in accord with the supposition expressed above. To remove the influence of this selection on the relation of milk yield to duration of intrauterine development it is necessary to resort to the partial correlation method. In other words we desire to measure the influence of pregnancy on yearly milk yield for a constant milk yield in the second month of lactation—third month of lactation—fourth month of lactation. The third column of table 2 gives the necessary data.

TABLE 3

Correlation between yearly milk yields and duration of time the calf is carried for a constant monthly milk yield

MONTH OF CONSTANT MILK YIELD	PARTIAL CORRELATION COEFFICIENT	CORRELATION COEFFICIENT ÷ PROBABLE ERROR
Three years to three years six months		
Second	-0.151 ± 0.028	5.4
Third	-0.112 ± 0.028	4.0
Fourth	-0.099 ± 0.028	3.5
Three years six months to four years		
Second	-0.183 ± 0.031	5.9
Third	-0.167 ± 0.031	5.4
Fourth	-0.145 ± 0.031	4.7

It will be noted that the correlation coefficient between the production of one month and the production of a subsequent yearly record is high. Such being the case it is clear that the selection of cows to remain barren on the basis of their monthly record being high will automatically increase any correlation that may exist between the yearly milk yield and the duration of time that the cow carries her calf. This disturbing influence can be removed, made constant, through the use of partial correlation coefficients. The correlation coefficients between yearly milk yields and duration of time the calf is carried with the monthly milk yield constant are given in table 3.

Table 3 shows that even when the effect of the selection based on the monthly milk yields is accounted for there remains a significant correlation between the length of time the calf is carried and the milk yield of the mother. It will be noted that the relation becomes less as the months of lactation advance. Such a result shows that the dairyman places more dependence on the third month's production than he does on the second as an indicator of the probable yield of the cow. Still more emphasis is placed on the fourth month than is placed on the third. Such being the case it appears more reasonable to determine the effect of carrying the calf on the milk yield of the fourth month's record as this month will remove most of the effect of the dairyman's conscious selection. From the known relations of the partial correlation coefficients it is possible to form the necessary equations. These are given below.

EQUATIONS TO PREDICT YEARLY MILK YIELD FROM MONTHLY MILK YIELD AND DURATION OF PREGNANCY

Three years to three years six months old.

$$\textit{Yearly milk yield} = 2210 - 87.98\,\textit{months calf carried} + 7.902\,\textit{milk yield fourth month} \quad (1)$$

Three years six months to four years old.

$$\textit{Yearly milk yield} = 2717 - 69.83\,\textit{months calf carried} + 7.688\,\textit{milk yield sixth month} \quad (2)$$

Solving these equations for the effect of carrying the calf on the milk yield of the mother we find that a cow carrying her calf nine months vs. one which is barren reduces her milk flow by the following amounts depending on the equation used: (1) 342 pounds of milk; (2) 628 pounds of milk.

If this value is converted into therms (therm is taken to be 1000 large calories as defined by Henry in "Feeds and Feeding" and not therm equaling small calorie of the Condensed Chemical Dictionary) of energy it is found that the bovine fetus during gestation uses up approximately 125 to 200 therms of energy considering that a pound of milk represents 337 calories (1).

These figures for the requirements of the bovine fetus are three to four times as large as those of Eckles (2). The difference in

methods of solving the problem seems to be responsible for the difference in results. Thus Eckles calculates the requirements of the fetus on the basis of its chemical composition in relation to the energy required to produce milk of similar composition. In the writer's view this should lead to a value which is too low as the living fetus would constantly increase its energy waste as its volume increased.

The results of Brody, Ragsdale and Turner (3) tend to confirm these conclusions although there is some question as to the ages of the cows dealt with in their work and whether or not any attempt was made to correct the results for any of the extraneous variables which might influence them.

In their essential conclusions the results of the three methods are not very far different. The average milk yield of three and one-half year old Guernsey advanced registry cows is 9100 pounds. On the basis of 500 pounds of milk as the requirement of the fetus the fetus requires for nutriment about 5 per cent of the average milk yield at this age. The range of milk yield for the same aged cows is from 4000 to 18,000 pounds or 14,000 pounds. The fetus requires for its development only 3 per cent of this range. The amount of energy required by the fetus is consequently but a very small part of the energy required for milk yield. Compared with the energy the cow is able to convert, the drain of the fetus on the cow is small.

Incidentally it is of interest to note that the severity of the effect of carrying the calf on the milk yield of the mother seems to increase slightly as the cow nears the time of parturition. The differences, for the cows aged three years and three years six months, while not very large, are probably significant.

SUMMARY

This paper presents a study of advanced registry Guernsey records to determine the effect of carrying the calf on the milk yield of the mother during that pregnancy. The data show that the carrying of the calf is slight but significant drain on the mother's milk producing capacity amounting to from 400 to 600

pounds of milk with an energy value of about 125 to 200 therms. The carrying of the fetus has no influence on the butter-fat percentage.

REFERENCES

(1) Woods, C. D., and Merrill, L. H. 1899 Cereal breakfast foods. Ann. Rept. Maine Agric. Exp. Sta. for 1899, pp. 92–106.

(2) Eckles, E. H. 1916 The nutrient required to develop the bovine foetus. Mo. Agr. Exp. Sta. Research Bull. 26, pp. 1–36.

(3) Brody, S., Ragsdale, A. D., and Turner, C. W. 1923 The effect of gestation on the rate of decline of milk secretion with the advance of the period of lactation. Jour. Gen. Physiol., v, no. 6, 777–783.

IV. SODIUM HYPOCHLORITE

THE PHENOL COEFFICIENT AND RELATIVE DISINFECTING POWER OF SODIUM HYPOCHLORITE[1]

HARPER F. ZOLLER AND SYLVIA M. EATON

Research Department of The Nizer Laboratories Company, Detroit, Michigan

Received for publication April 20, 1924

Authentic data on the relative disinfecting action of sodium hypochlorite is very sparse. Pusch (1) reported the results of some generalized experiments in 1908. While his work dealt in the use of electrolytic bleach liquors of variable content of alkalinity, he expresses his data in the concentration of chlorine per liter of solution, and thus establishes definite points for check measurements. It is noteworthy that this data is of little value to us in our present attack of the problem, because Pusch dealt primarily with organic infested media, such as sewage, blood clots, and profuse cultures of a few bacteria. Sayer and Patty (2) record the results of some tests with sodium hypochlorite wherein infected objects (soda fountain utensils) are immersed for certain periods, and the sterilizing action noted. Dakin and Dunham (3) have given some empirical data of similar value obtained from the use of certain commercial, chlorine derived products.

The more refined methods of examining disinfectants[2] as suggested in the Rideal-Walker, Lancet Commission of Disinfectants,

[1] This paper was read at the annual meeting of the Society of American Bacteriologists, December, 1922, at Detroit, Mich.

[2] There is nothing in the terminology or method of the "phenol coefficient" which implies any specific action of the disinfectant when applied in practice. If we are careful to avoid interpreting the results of a phenol coefficient determination as being directly commensurate with the disinfectant's power in all kinds of products under any set of conditions, without any regard for the chemical structure and properties of the disinfectant or the disinfectant medium, we can still look upon the phenol coefficient as a useful biological meter. It was devised to measure the relative distinctive action of chemicals upon bacterial growth (reproduction) under a given set of conditions. The most insiduous opponent of this test cannot do other than agree in general with the relative coefficients of phenol vs. mercuric chloride, phenol vs. thymol, phenol vs. boric acid. These bear out in practice the general order of the determined coefficients.

or the United States Hygienic Laboratory Method, have not been applied in those mentioned above. No regard for the reaction (pH) of the disinfecting solution, or its chance effect upon the disinfecting power, is apparent in the work.

SCOPE OF THE PRESENT STUDY

First: We have attempted to employ the Hygienic Laboratory Method (4) with only slight modification in our study of sodium hypochlorite disinfection. The modification is as follows:

a. Substitution of 0.3 per cent $Na_2HPO_4 \cdot 2H_2O$ and 0.2 per cent NaCl in lieu of 0.5 per cent NaCl in the culture medium to buffer the reaction between 6.9 and 7.1 pH.

b. The expression of all master dilutions and final dilutions of disinfectants in percentage strength.

The use of typhoid bacilli as a criterion of disinfecting power is open to criticism, since the vitality of bacterial cells, in general, is not characterized by any particular species. But for the sake of relative data alone we may as well choose the typhoid bacilli for the purpose of comparing one disinfectant with another, under like conditions. We have therefore determined the phenol coefficient, within reasonable limits, of sodium hypochlorite using a virile culture of typhoid (Hopkin's) organisms. The original cultures used in this investigation were furnished through the courtesies of the Digestive Ferments Company, of Detroit, and the Army Medical Museum, of Washington.

Second: Sodium hypochlorite, in dilute and concentrated solutions, acts in a dual rôle towards organic matter. It is (*a*) a strong chlorinating substance, and (*b*) a strong oxidizing substance. The former is exhibited in solutions of low pH (below pH 8.0), and the latter at higher pH (above pH 9.0). In any event its solutions are distinctly on the side of a very strong oxidizing potential. In this respect sodium hypochlorite differs markedly from most common disinfectants. We would postulate a different type of disinfectant action from it upon bacterial cells. Mercuric chloride combines with and coagulates the protein of the protoplasm. The phenols are supposed to act toxically to the cellular functions, but probably not as a combining agent

in the common view. Chloroform and kindred substances probably anesthetize, whatever this may signify. Formaldehyde actually combines with the protein and like material of the cell. Hydrogen peroxide disinfects by furnishing an atmosphere of oxygen in the vicinity of the normal reducing centers of the cells, and thereby opposes their functions.

Sodium hypochlorite may act in the dual rôle simultaneously upon the living cell. The bound chlorine may actually chlorinate certain compounds within the cell. Or the "available chlorine" (oxygen) may act in the capacity of hydrogen peroxide. Now it is conceivable that bacterial cells of certain species will tolerate more chlorine or oxygen than others. Gillespie (5) has shown that bacteria which liberate free hydrogen, and some which do not, maintain a very high reduction potential. Measurements by Clark have confirmed the finding. Naturally coli bacilli, which readily evolve hydrogen in carbohydrate media, should establish a very strong reducing medium, both within and without the cell. We would expect this bacterium to consume more of a disinfecting agent like sodium hypochlorite or hydrogen peroxide than an organism of the prodigiosus family. In commencing the study this factor was kept in the foreground, and the phenol coefficient was determined within rather wide limits upon a number of organisms: coli (Neapolitanum), typhoid (Hopkins), proteus vulgaris, alkaligines, gonococcus, anthracis, and tubercle bacilli.

Third: The relative disinfecting power of NaOCl and mercuric chloride on B. coli was determined by the same method used in the phenol coefficient study. The results are expressed as the mercuric chloride coefficient of NaOCl.

Fourth: Feeling that the hydrogen ion concentration of the diluted disinfectant, into which the 0.1 cc. of virile culture is measured, might exert an appreciable influence upon the phenol coefficient, we set out to discover its magnitude. Since the sodium hypochlorite we used furnished in the final dilutions a pH range of 9.72 to 9.83, a quantity of M/10 buffer was prepared which consisted of Clarke and Lub's borate-KCl mixture. When diluted to M/20 this mixture had a pH of 9.90. It served as a

buffer for the phenol, and held the pH of the latter in the dilutions used to pH 8.8 to 9.1. The influence of the buffer mixture itself upon the growth of B. coli (Bact. Neapolitanum) was predetermined under the conditions of the experimentations.

EXPERIMENTAL PROCEDURE

Reagents

The phenol used in this study was a quantity of Malinkrodt's c.p. which we redistilled from an all glass still. The first and last fractions were discarded. The standard 5 per cent solution was standardized against 0.1 N $Na_2S_2O_3$, following the bromate bromide procedure.

Mercuric chloride was obtained from Merck, "tested purity" and was recrystallized from conductivity water.

Sodium hypochlorite was prepared according to the method proposed by one of us (6). Pure chlorine from a drum was conducted into a solution consisting of 5 per cent of NaOH and 2.5 per cent of anhydrous Na_2CO_3 until the final concentration of "available chlorine" was 2.0 per cent. This solution was kept throughout the entire series of studies, which lasted during three months. It was not necessary to screen the hypochlorite solution from the light. A master dilution was made up from this 2.0 per cent solution which exacted 0.25 per cent of "available chlorine." The pH of the master dilution approached 10.1. This master dilution was made fresh preceding each disinfecting set.

The boric acid, NaOH and KCl were purified according to the well known procedure of Clark and Lubs.

Thermostats

The temperature for holding the disinfecting solutions at transfer was maintained at 20°C., ±0.1 in a circulating water bath electrically controlled.

A Freas electrically controlled incubator was used for incubating the inoculated broth tubes. A 37.5°C. temperature was maintained for all organisms whose optimum growth lay in this region.

Cultures of organisms

It was mentioned that pure cultures of these organisms had been obtained from reliable sources. Transfers of these organisms to sterile agar slopes were made every two weeks. A tube of the standard culture medium recommended by the Hygienic Laboratory (with our modification) was inoculated with the culture of the specific organism three days before the disinfection study, and another transfer was made twenty hours before using. It should be stated here that Bacto peptone (Difco) was substituted throughout for Armour's product.

The medium for the tubercle bacilli was composed of: 1 per cent Bacto peptone; 0.2 per cent NaCl; 0.3 per cent $Na_2HPO_4 \cdot 2H_2O$; 0.5 per cent of lipoid medium (supplied by Mr. Dunham of the Digestive Ferments Company, which courtesy is hereby acknowledged). Inasmuch as this organism is a surface grower, possessing a waxy coat, it is essential that all clumps be broken up and brought into a suspension. The cultures before transfer were therefore forty-eight hours old, and the tubes thoroughly shaken by tapping while being held between the thumb and forefinger to serve as a rocker. Two or three minutes' tapping brought the culture into the form of a homogenous suspension. Transfer from the disinfecting tubes showed that the 0.1 cc. of tuberculosis culture carried a goodly supply of the living cells into the disinfectant. It may be said in passing that the tuberculosis series was a very beautiful experiment in disinfection, both from the standpoint of sharp detail and esthetics.

The anthrax cultures were treated in the same manner as the tubercle bacilli, but were grown in the standard medium.

All other cultures, typhoid, coli, proteus, alkaligines and gonococcus, were treated in the regular manner. The gonococcus was grown in the same type of medium used for the tubercle organisms.

EXPERIMENTAL RESULTS

We have remarked that the limits of dilution chosen yielded in the majority of instances a phenol coefficient of large magnitude. In fact it is much greater in value than any previously

quoted. Repeated checks on the phenol coefficients of each organism agree in all detail so that the values expressed herewith are considered consistent with the dilutions and conditions used. By choosing intermediate dilutions and calculating interpolated phenol coefficients, it can be seen that the magnitude of the coefficient can be reduced about 20 per cent the order would not be changed.

TABLE 1

Phenol coefficient of NaOCl. Set No. 6 organism typhoid (Hopkins) pH 7.4 of culture

Date July 24, 1922. Checked July 27, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
			Phenol		
per cent					
2.5	−	−	−	−	−
2.0	−	−	−	−	−
1.5	+	+	−	−	−
1.0	+	+	+	+	+
0.5	+	+	+	+	+
			NaOCl		
0.010	−	−	−	−	−
0.0075	+	−	−	−	−
0.0050	+	+	+	+	−
0.00375	+	+	+	+	+

Calculations for coefficient = $\dfrac{\dfrac{2.9}{0.01} + \dfrac{1.5}{0.0075} + \dfrac{1.5}{0.005}}{2} = 233.3$

By way of illustration we are giving herewith the results of a single test to show how the dilutions and time of exposure to disinfectant relate.

It is apparent that if we had made dilutions of NaOCl intermediate between 0.0075 and 0.005 per cent we could have obtained a better slope of rate in our table. The same may be said of the phenol dilutions. It is doubtful if this is very important in fixing the relative power of disinfection.

TABLE 2

ORGANISM	PHENOL COEFFICIENT (APPROXIMATELY)
Tubercle bacilli	42.8
Alkaligines	100
Anthracis	160
Bact. Neapolitanum (coli)	200
Gonacoccus	280
Proteus	330

TABLE 3

Disinfecting power of M/20 *borate potassium chloride buffer Organism—Bact. Neapolitanum (coli)*

Temperature 20°C. pH, 9.91. July 31, 1922. August 1, 1922.

DILUTION BUFFER	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
M/20	–	–	–	–	–

TABLE 4

Influence of H ion concentration upon the phenol coefficient of NaOCl. Organism Bact. Neapolitanum

pH of culture, 7.4. Temperature 20°C. August 1, 1922. Concentration of buffer in final dilutions M/20 (borate—KCl).

DILUTION	TIME OF EXPOSURE					pH ELECTROMETRICALLY
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes	
Phenol in buffer						
2.0	–	–	–	–	–	8.80
1.5	–	–	–	–	–	8.98
1.0	+	+	+	+	+	9.08
0.5	+	+	+	+	+	9.12
NaOCl						
0.0075	–	–	–	–	–	9.83
0.005	+	+	+	+	–	9.76
0.004375	+	+	+	+	+	9.73
0.00375	+	+	+	+	+	9.72

Coefficient = 200 (approximately).

The phenol coefficients as determined for the other organisms are shown in table 2.

The pH of the diluted hypochlorite solutions used in the exposure tubes lay between 9.5 and 9.8. That of the phenol pH 7.0 to 7.2.

The points were determined colorimetrically after checking the buffer comparison mixtures electrometrically. Just how great an influence this great difference in hydrogen ion concentration exerts upon the disinfecting ratio of NaOCl and phenol

TABLE 5

Phenol coefficient of NaOCl without buffering the phenol. Organism Bact. Neapolitanum

pH culture, 7.4. Temperature 20°C. July 27, 1922.

DILUTION	TIME OF EXPOSURE					pH COLOR
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes	
Phenol						
2.0	−	−	−	−	−	7.0
1.5	−	−	−	−	−	7.1
1.0	+	+	+	+	−	7.2
0.5	+	+	+	+	+	7.2
NaOCl						
0.0075	−	−	−	−	−	9.8
0.005	+	+	+	+	−	9.75
0.004375	+	+	+	+	+	9.7
0.99375	+	+	+	+	+	9.7

Calculated coefficient (phenol) = 200 (approximately).

can be seen from the results given in table 3. We chose to work on Bact. Neapolitanum because of the large phenol coefficient observed and the ease of propagation of this organism. The buffer is not toxic to the above organism in the concentration employed.

It would seem that the pH of the disinfectant solution throughout the ranges employed has practically no effect upon the vitality of the living cell during this short exposure to its action.

TABLE 6

Relative disinfectant action of $HgCl_2$ and NaOCl Organism Bact. Neapolitanum

pH of culture, 7.5. Temperature 20°C. August 3, 1922. Checked August 4, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
			$HgCl_2$		
0.025	−	−	−	−	−
0.01	+	−	−	−	−
0.0075	+	+	+	−	−
0.0050	+	+	+	+	+
			NaOCl		
0.0075	−	−	−	−	−
0.0050	+	+	−	−	−
0.004375	+	+	+	+	−
0.00375	+	+	+	+	+

Calculated $HgCl_2$ coefficient = 2.3 (approximately).

TABLE 7

Phenol coefficient of NaOCl on tubercle bacilli. Organism tubercle

Age of culture, forty-eight hours. Temperature 20°C. Time of incubation at 37.5° of transfer, sixty hours. December 12, 1922. Checked December 8, 1922.

DILUTION	TIME OF EXPOSURE				
	5 minutes	7.5 minutes	10 minutes	12.5 minutes	15 minutes
			Phenol		
2.0	−	−	−	−	−
1.5	+	−	−	−	−
1.3	+	+	+	+	−
1.15	+	+	+	+	+
			NaOCl		
0.05	−	−	−	−	+
0.035	−	−	−	+	−
0.025	+	+	+	+	+
0.015	+	+	+	+	+

Calculated coefficient = 42.8 (approximately).

The mercuric chloride coefficient of NaOCl

When $HgCl_2$ is used in place of phenol on Bact. Neapolitanum we observe that sodium hypochlorite is slightly superior. The results of a preliminary series with these two substances gave the results in table 6.

Effect of a wax capsule on the disinfectant action of NaOCl

Virile forty-eight-hour cultures of tubercle bacilli were transferred to phenol and NaOCl exposure tubes at 20°C. The lipoid medium was used.

It is evident that there is a growth lag in case of the tuberculosis and sodium hypochlorite. It may be that the apparent growth lag is really a case of irregular quantities of the exposed material obtained by means of the platinum wire loop (spiral) at the time of transfer.

DISCUSSION OF RESULTS

While the coefficients obtained are large we feel that they are indicative of the phenol coefficient of sodium hypochlorite. We trust that some will be tempted to prepare sodium hypochlorite in accordance with our method, and repeat these experiments. The value of sodium hypochlorite has been both overestimated and underestimated. We actually need much scientific data on the subject. In the paper which follows we have shown that under certain conditions sodium hypochlorite is almost valueless as a bactericidal agent (i.e., in high protein solutions such as milk), yet even here it works with tremendous rapidity.

Our results seem to show that there may be some relationship between the reduction potential of bacterial cells and the toxic action (bactericidal) of sodium hypochlorite. But this needs further confirmation. One can expect that if there is a quantity of reducing material carried into the exposure tubes by the 0.1 cc. of virile culture, that such would require a comparatively large quantity of NaOCl to oppose it—to oxidize it. Likewise, if much protein material is carried over it would require much NaOCl for chlorination. However, the use of the same medium for each organism, and carefulness of transfer measurements,

should render this factor quite constant. Reduction potentials of the cultures used have been measured with a bare gold electrode, according to the method of Clark; likewise, reduction potentials of the exposure solutions have been measured, but these will not be discussed at this time. It is only in dilution experiments that any such exo-cellular or endo-cellular reduction effects can be determined.

The influence of small changes in pH on the phenol coefficient of sodium hypochlorite is not important in face of such widely different types of disinfectants.

From the standpoint of promiscuous use, sodium hypochlorite is a far better and safer disinfectant for the pathological laboratory, hospital and clinic than mercuric chloride.

We have become accustomed to the use of antiformin (equal parts of 15 per cent NaOH and dilute NaOCl) for tuberculosis detection. The fact that NaOCl has such a high phenol coefficient on tuberculosis organisms requires some comment. Doubtless the action of antiformin is largely dependent upon the strong alkalinity of the solution. The alkali serving to dissolve and hydrolize the protein matter in the sputum. The small quantity of NaOCl merely acting as catalytic agent in the sense of hastening the action of the alkali on the bacterial cells. The tubercle cell, because of its thick covering of wax, resists the action of the alkali for a short period. Smears containing tuberculosis organisms and sputum were treated with 15 per cent NaOH and then stained as in the antiformin method. Other smears were prepared containing the regular antiformin mixture and with NaOCl in varying proportions. The results showed that the dissolving action of antiformin is primarily due to the free alkali. The presence of the NaOCl actually shortened the time of solution. Likewise the hypochlorite must actually kill many of the tuberculosis organisms, although some may survive the treatment. It is not likely that any other organisms in the sputum could survive the treatment with antiformin.

SUMMARY

1. The phenol coefficient of stable sodium hypochlorite is very high, varying between 42 and 300 with the organisms employed.

2. The hydrogen ion concentration of the disinfectant in the exposure tube has little or no influence upon the phenol coefficient of NaOCl throughout the ranges employed.

3. Mercuric chloride is not as good a bactericidal agent as sodium hypochlorite.

4. NaOCl has a high phenol coefficient on the tubercle organism. The value of antiformin depends largely upon its content of alkalinity.

REFERENCES

(1) Pusch, H.: Experimental researches into the properties of electrolytic bleaching liquors. Centr. Bakt., Parasit., and Infekt., xlvi, Sect. 6 (1908).

(2) Sayer, L. E. and Patty, F. A.: Kans. State Board Health Bul. 16, 76–80 (1920).

(3) Dakin and Dunham: Handbook of Antiseptics.

(4) Disinfectant testing by the Hygienic Laboratory Method. Reprint No. 675. Public Health Reports, July 8, 1921. United States Public Health Service.

(5) Gillespie, L. J.: Reduction potentials of bacterial cultures, etc. Soil Sci., ix, 199 (1920).

(6) Zoller, H. F.: The preparation of concentrated sodium hypochlorite solutions of great stability, etc. Jour. Ind. Eng. Chem., xv, 845 (1923).

THE BACTERIAL CONTENT OF ICE CREAM[1]

A. C. FAY AND N. E. OLSON

Kansas Agricultural Experiment Station, Manhattan, Kansas

Received for publication February 20, 1924

INTRODUCTION

The phenomenal development of the ice cream industry is shown by statistics recently released by the Dairy Division of the United States Department of Agriculture. The total value of ice cream produced has increased 281 per cent in the seven-year period, 1914 to 1921. The ice cream made in 1914 was valued at $55,983,000 and in 1921 at $213,262,000. The consumption of ice cream in the United States in 1909 was 80,000,000 gallons, and in 1920 there were 260,000,000 gallons consumed, an increase of 225 per cent. This large increase is no doubt due to a better appreciation of its high nutritive value as well as its cooling effect.

With the increased demand for ice cream, there have come modern methods of manufacture and control. Laws have been passed in several states regulating the percentage composition and in some instances requiring pasteurization of the ice cream mix. A few attempts have been made to regulate the bacterial content of ice cream. This has not been entirely successful due to the limited knowledge of what a fair bacterial standard for ice cream ought to be. Unlike butter and cheese, ice cream is not so dependent upon bacteria for its flavor, and, except for the use of starters in recent years, microörganisms apparently have no function in the manufacturing process. This fact, together with the low temperature which practically inhibits bacterial growth, has led to the more or less prevalent but erroneous idea that bacteria are of no concern to the ice

[1] Contribution No. 61 from the Department of Bacteriology and No. 48 from the Department of Dairy Husbandry of the Kansas Agricultural Experiment Station.

cream maker. On the contrary the writers believe that the number of bacteria in ice cream is of vital concern both to the manufacturer and the consumer. As will be pointed out later, ice cream that has been made from clean raw products, and properly handled will contain relatively few bacteria. On the other hand ice cream that has been made from a poor grade of raw products, in unclean utensils, or has been improperly cared for during the manufacturing process will contain many bacteria and is undesirable from a sanitary point of view.

One of the most satisfactory methods of demonstrating the care with which ice cream has been produced is by determining the number of bacteria in it. When ice cream is found to contain 50,000,000 bacteria per gram, it may be safely assumed that it is not a desirable product to serve to sick people, to children, or even to healthy adults. An exception to this would be found in ice cream that has been ripened with a pure culture starter. On the other hand ice cream that contains 5000 bacteria per gram has more than likely been produced under carefully controlled conditions and from the best grade of raw products obtainable. The bacterial count, therefore, serves to differentiate between these two extremes. It is easy enough to say that ice cream containing 50,000,000 bacteria is undesirable, and one containing 5000 bacteria per gram is desirable from a sanitary standpoint, but it is not so easy to determine at what point between these two extremes a line of demarcation should be drawn. The present status of what is known about the bacterial content of ice cream does not justify the establishment of such a standard.

REVIEW OF LITERATURE

Although ice cream was probably first made about 1750 in Naples, it has not received attention by bacteriologists and public health officials until recent years.

In 1907, Pennington and Walter (18) reported a bacteriological study of commercial ice cream in Philadelphia. They classified 60 ice cream plants as follows: clean, 20; fair, 26; dirty, 6; and filthy, 8. They found a definite relation between the

cleanliness of the plant and the total number of bacteria in the finished product, but no relation between cleanliness and the number of streptococci. The maximum number of bacteria found in this investigation was 151,200,000 per cubic centimeter, and the minimum 50,000 per cubic centimeter. In one plant in which extraordinary sanitary precautions were taken, the bacterial counts of the finished ice cream in three successive trials were 6,535,000, 33,120,000 and 20,550,000 respectively.

In the same year Stiles and Pennington (23) examined 263 samples of ice cream in Washington, D. C., and found an average of 26,000,000, a maximum of 365,000,000 and a minimum of 137,500 bacteria per cubic centimeter. An investigation of 53 ice cream manufactories in Washington showed that 62.2 per cent were making ice cream in cellars and basements which were unfit for the production of such food. The 53 plants were classified as follows: clean 5.6 per cent; dirty 35.8 per cent; fair 30.1 per cent; and filthy 16.9 per cent. These authors believe that the cream and milk supply must be bettered before much improvement can be expected in the number of bacteria in ice cream.

Wiley (25) states that bacterial standards should be devised which would "exclude from sale ice cream containing a bacterial flora of the enormous proportions exhibited by some of the samples examined."

Buchan (5), in 1910, made a study of 50 ice cream venders (33 English and 17 Italian) and found "that most of the ice cream was not produced under sanitary conditions." Buchan's investigation involved an inquiry into the methods of preparation of the ice cream, a sanitary inspection of the premises, and a bacteriological study of the mix at various stages in the manufacturing process. He classed 10 per cent of the premises as clean, 36 per cent as fair, 46 per cent as dirty, and 8 per cent as filthy. The average bacterial count for 38 samples was 372,000,000 per cubic centimeter, the lowest count for 50,000 and the highest 3,800,000,000. Five of the samples, or 13 per cent contained over 1,000,000,000 organisms per cubic centimeter. Buchan concluded from his study that ice cream should

not be offered for sale if it contained more than 1,000,000 organisms capable of growing on nutrient agar (reaction) + 1 per cent at 35° to 37°C. in two days. This standard is quite lenient when compared to the requirements established by the Board of Public Health of the State of Victoria (22) in 1906, that ice cream shall contain not more than 50,000 organisms per cubic centimeter.

Newman (16) has pointed out that the frequent recurrence of epidemics due to ice cream in English cities led to the establishment of strict regulations by Liverpool in 1895, London in 1904 and by Glasgow in 1905. At the present time the city of Boston maintains a bacterial standard of 1,000,000 per cubic centimeter for ice cream.

In 1914, Bahlman (3) reported the results of investigations relating to ice cream in Cincinnati. An examination of 67 samples in 1911 showed a minimum of 200,000 and a maximum of 400,000,000 bacteria per cubic centimeter. In 1914, after the passage of an ordinance in Cincinnati requiring pasteurization of the cream, bacterial counts on 39 samples of ice cream showed a minimum of 60,000, and maximum of 35,000,000 per cubic centimeter. By pasteurizing the ice cream mix at 143° to 146°F. for thirty minutes, and carefully cleaning and steaming all utensils, Bahlman was able to produce ice cream containing no more than 12,000 bacteria per cubic centimeter. He concluded that if ice cream is properly pasteurized it would be very easy to comply with a legal standard limiting the bacterial content to 500,000 per cubic centimeter.

Ayres and Johnson (1) in 1915 collected 91 winter samples and 94 summer samples of ice cream from retail stores in Washington. None of these samples contained less than 100,000 and only 9.5 per cent contained less than 500,000 bacteria per cubic centimeter. The average for the 185 samples was 37,000,000; the maximum 510,000,000 and the minimum 120,000 per cubic centimeter.

In 1917 Hammer (10) made a comprehensive study of ice cream, including an investigation of the source of bacteria, the possibilities of securing a low bacterial count, and

the changes in the bacterial flora that take place during storage at low temperature. He concluded that unpasteurized cream is the greatest source of bacteria, that gelatin may or may not be responsible for large numbers, and that sugar, and vanilla extract are unimportant as a source of contamination. His investigations, also showed the importance of the utensils as a source of bacteria. By pasteurizing cream at 60°C. for twenty minutes, and sterilizing all utensils in an autoclave, he was able to produce ice cream in 15 and 20 gallon lots containing as few as 4200 bacteria per cubic centimeter.

Hammer and Goss (11) examined 13 samples of ice cream, other than vanilla, and found the bacterial counts to range between 130,000 and 40,000,000 per cubic centimeter. Water-ices and sherbets were found to contain from 6 to 6800 bacteria per cubic centimeter.

Ayers and Johnson (2) in 1917, made a study of the accuracy of bacterial counts on ice cream. By removing samples from nine places in various parts of a one-gallon container they were able to obtain results that checked very well. The bacterial counts on 198 samples ranged from 7150 to 225,000,000 per cubic centimeter. Fifteen samples contained more than 1,000,000, 5 more than 50,000,000, 9 more than 25,000,000 and 3 in excess of 100,000,000 bacteria per cubic centimeter.

In 1921 Hammer (13) emphasized the importance of the employees in an ice cream plant as a potential source of contamination with pathogenic organisms.

Jordon (14), in 1922 reported on the bacteriological examination of 1940 samples of ice cream in Boston, covering a period of ten years. Approximately 40 per cent of the samples contained more than 1,000,000 bacteria per cubic centimeter.

In 1923 one of the writers (9) reported on 115 samples of ice cream, all of which has been pasteurized. The maximum count was 47,000,000, the minimum 1500, and the average 1,895,000 per grams. Approximately 9 per cent of the samples contained fewer than 5000, 50 per cent less than 100,000, and 85 per cent less than 1,000,000 bacteria per gram.

Since the preparation of this manuscript Fabian and Cromley (26) have published the results of their study of the influence of manufacturing operations on the bacterial content of ice cream. Their method of attack and the results obtained are remarkably concurrent with the findings reported in this paper.

EXPERIMENTAL

The purpose of the experiment of which this paper is a partial report is to study the factors affecting the bacterial count of ice cream, the possibilities of producing ice cream having a low bacterial content under commercial conditions, and ultimately to establish a bacterial standard. The principal work so far has related to the possibilities of producing ice cream of a low bacterial count. The investigation was carried on in a representative commercial plant rather than in the college laboratories. About 200 gallons of ice cream were made per day during the summer, the amount varying between 100 and 400 gallons. The equipment, the surroundings, the methods employed, and the source of raw materials were such as will be found in many small ice cream plants throughout the country. For this reason the results should be applicable to every day conditions as they are found in the average commercial plant.

Plant methods. The mixing vat used was a plain cream ripener of 300 gallon capacity. The vat was washed first with cold water, then with hot water and Wyandotte washing powder, and finally rinsed with hot water. Following this it was steamed with covers closed until the temperature reached 200°F. or over. It was then allowed to stand with covers closed for ten minutes. The aging vat, also a 300-gallon cream ripener, was washed and sterilized in the same manner as the mixing vat.

The homogenizer was thoroughly washed and steamed before and after using in about the same manner as the mixing vat. The dissembled parts, after washing and steaming, were protected from dust, and steamed again for five minutes when the machine was reassembled.

The freezer was washed with cold water to rinse out the adhering ice cream and to partially warm the freezer. This was

followed by two washings of hot water, the last one containing Wyandotte washing powder. The machine was then rinsed with clear, hot water and sterilized by steaming for five minutes with the hose entering the outlet gate.

Preparation of the mix. In preparing the mix the liquid ingredients including milk, cream, and water were placed in the vat together. The gelatin, skim milk powder, and sugar, in the dry powder form were added separately by sprinkling over the surface of the cold liquid. Steam was then turned on and the contents of vat vigorously agitated until thoroughly mixed.

Since the unsalted butter could not be mixed properly with the other ingredients at ordinary temperatures it was necessary to sample the butter and the mix without the butter separately in order to determine the bacterial count of the mix before pasteurization. It was found that the skim milk powder, gelatin and sugar were practically all in solution or suspension by the time the mix reached 110°F., and a fair sample could be obtained. This temperature for the short time involved (about ten minutes) was not considered sufficient exposure to affect the bacterial content materially. The butter in pieces no larger than 1 pound was added immediately after this sample was taken from the vat. By the time the mix reached 140°F. all the butter was melted and mixed with the liquid constituents. The bacterial count before pasteurization, therefore, is a calculated count. It is calculated from the bacterial count of the butter, together with the number of pounds used, added to the bacterial count of the mix excluding the butter.

Pasteurization. The mix was pasteurized in most instances at 150°F. for thirty minutes. In some cases other combinations of time and temperature were used for comparison. The pasteurizing vat and also the aging vat were equipped with a Tycos bi-record recording thermometer.

Homogenization. After the mix left the pasteurizing vat, it was homogenized at pasteurizing temperature by means of a Viscolizer, at 1500 pounds pressure.

Aging. From the Viscolizer the mix was pumped into the aging vat to be cooled. When it had cooled to about 70°F. a

ripening powder was added. The temperature of the mix was then reduced to below 40°F. (usually about 36°F.) and allowed to stand over night. During the aging process the temperature remained between 36°F. and 45°F. for six to eight hours and then gradually increased to 50°F. after ten to fourteen hours. Rarely did the thermometer register above 50°F. the next morning. In a few instances the aging process was extended to thirty-six or forty-eight hours. In these cases it was kept below 50°F. throughout the period.

Freezing. The freezer in use was a 40-quart Fort Atkinson brine freezer. A sample was taken from the freezer and stored for two days in a hardening room, maintained at a temperature between 0 and 8°F. Another sample was taken simultaneously for immediate analysis.

Bacteriological methods. The bacteriological analyses were made on a gravimetric basis. The purpose of using a gravimetric sample was to overcome the variation in volumetric samples of melted ice cream resulting from incomplete exhaustion of air, and the adherence of melted ice cream to the pipettes. In order to make all the counts comparable a 10-gram sample was weighed under aseptic conditions for all of the samples as well as for the ice cream samples. The counts, therefore, represent the number of bacteria *per gram.* The determination of total numbers of bacteria was made according to the plate method recommended by the American Public Health Association (21), Liebig's beef extract, and Difco peptone were used in the preparation of the agar.

An effort was made to determine the different kinds of organisms present. Serial dilutions were made by inoculating a series of tubes with dilutions ranging from 1 in 10 to 1 in 10,000,000. One set of Durham fermentation tubes containing one per cent lactose broth and brom-thymol-blue as an indicator were inoculated. Another series of tubes containing nutrient gelatin was also inoculated. The highest dilution showing acid and no gas in lactose broth after forty-eight hours at 37°C. was recorded as the number of acid producing types. Similarly, the highest dilution showing gas was recorded as the number of acid

and gas producing types. The gelatin tubes were also incubated forty-hours at 37°C., and then removed and placed in iced water. The highest dilution which failed to solidify was recorded as the number of liquefying types present. Obviously there is a very high per cent of error in results obtained from serial dilutions. The conclusions which may be drawn from data obtained in this manner must necessarily be very general.

Samples were taken throughout the routine of manufacture as follows: (1) from the liquid constituents of the mix, including cream, whole milk, and the water (if added); (2) the mix before pasteurizing, not including the butter; (3) the butter; (4) the mix after pasteurization and before homogenizing; (5) after homogenizing and before aging; (6) after aging fifteen to eighteen hours and before freezing; (7) from the freezer; and, (8) after two days storage in the hardening room. Other samples were taken at intervals of the gelatin, sugar, skim milk powder, etc. All liquid samples were taken with a sterile milk thief and placed in a sterile glass stoppered bottle. From the vats, samples were taken at eight different places to insure as representative a sample as possible. The butter and frozen ice cream were sampled with a sterile butter trier by removing a core from four or five places in the container.

EXPERIMENTAL RESULTS

Table 1 shows the total counts obtained from various steps in the process of manufacture. During the first eight runs the customs and practices of the plant were not altered. From the ninth to the twenty-eighth run the entire manipulation of the mix was supervised by the writers.

It will be observed that the bacterial counts of the mix before pasteurization are in many instances exceedingly high, there being ten cases in which it exceeded 10,000,000 per gram. The lowest count before pasteurization was 2,059,000 and the highest 67,500,000 per gram. In general, it may be seen that the bacterial count after the mix is pasteurized governs to a large extent the bacterial count of the finished product. The effect of the various manipulations during the manufacturing process will be taken up separately.

Pasteurization. Table 2 shows the counts before and after pasteurization, the decrease in numbers of organisms, and the

TABLE 1

The total bacterial counts at various stages of the manufacture of ice cream

EXPERIMENT NUMBER	MIX BEFORE PASTEURIZING (CALCULATED)	MIX AFTER PASTEURIZING	AFTER HOMOGENIZING	BEFORE AGING	BEFORE FREEZING AND AFTER AGING	AFTER FREEZING	AFTER HARDENING FORTY-EIGHT HOURS
1	12,031,000	170,000	233,000	120,000	18,500		
2	3,398,000	500,000	160,000	60,500	44,000	56,000	67,500
3	6,028,000	285,000	128,000	250,000	262,000	188,000	107,500
4	6,306,000	58,000	52,500	39,500	35,500	52,500	119,000
5	2,477,000	75,500	120,000	79,000	79,500	97,000	102,500
6	26,147,000	2,210,000	2,830,000	3,700,000	3,835,000	2,880,000	1,530,000
7	31,100,000	35,500	111,000	95,000	244,000	1,165,000	1,080,000
8	9,328,000	124,000	132,000	178,500	165,500	900,000	1,315,000
9	38,985,000	27,000	270,000	11,000	10,000	24,000	30,000
10	22,053,000	70,000	109,000	160,000	41,000	97,000	66,000
11	67,500,000	2,070,000	153,000	111,000	115,500	160,000	21,000
12	29,360,000	44,000	2,980,000	23,000	27,000	64,000	22,000
13	2,059,000	6,500	11,000	9,000	8,000	20,000	13,000
14	44,774,000	20,000	20,000	14,000	12,000	11,000	7,000
15	6,197,000	52,000	19,500	73,000	108,000	172,000	140,000
16	46,103,000	27,000	63,000	152,000	70,000	150,000	115,000
17	6,898,000	32,000	36,000	16,000	63,000	67,000	35,000
18	9,685,000	42,000	44,000	43,500	10,500	26,000	19,000
19	2,290,000	13,000	16,000	14,000	28,000	22,000	23,000
20	5,634,000	51,000	47,000	54,000	48,000	42,000	34,500
21	4,236,000	70,000	53,000	73,000	33,000	31,000	30,000
22	2,551,000	5,700	7,000	5,200	9,800	10,000	7,400
23	6,803,000	19,300	27,600	11,000	14,000	29,500	11,800
24	53,386,000	6,400	9,500	8,200	6,300	7,300	30,500
25	5,219,000	9,850	12,200	11,400	8,400	11,650	9,650
26	12,630,000	26,000	84,000	25,400	16,300	18,650	24,150
27		60,000	12,000	7,700	8,350	12,000	8,150
28	2,685,000	49,000	29,000	25,000	65,000	77,000	62,000
Average.	17,261,926	219,953	277,475	191,782	192,362	236,688	186,320*

* The average bacterial count of the finished product in experiments 2 to 8 inclusive was 617,357; the average for experiments 9 to 28 inclusive when more care was taken in the manufacturing operation was 35,432 bacteria per gram.

per cent efficiency of pasteurization. The last two columns show the time and temperature used in this process for each of the runs.

The average of the counts before pasteurization in the 28 experiments was 17,261,926 per gram, and after pasteurization 219,953 per gram. This gives an average pasteurization ef-

TABLE 2

The effect of pasteurization on the total number of bacteria in ice cream

EXPERIMENT NUMBER	MIX		PER CENT EFFICIENCY	PASTEURIZATION	
	Before pasteurization	After pasteurization		Temperature	Time
					minutes
1	12,031,000	170,000	98.587	140	20
2	3,598,000	500,000	86.104	147	30
3	6,028,000	285,000	95.273	143	30
4	6,306,000	58,000	99.081	142	30
5	2,477,000	75,500	96.952	144	25
6	26,147,000	2,210,000	91.548	144	25
7	31,100,000	35,500	99.886	144	25
8	9,328,000	124,000	98 671	146	25
9	38,985,000	27,000	99.931	145	25
10	22,053,000	70,000	99.682	143	30
11	67,500,000	2,070,000	96.933	150	30
12	29,360,000	44,000	99.852	148	30
13	2,059,000	6,500	99.685	148	30
14	44,774,000	20,000	99.956	152	30
15	6,197,000	52,000	99.161	150	30
16	46,103,000	27,000	99.942	151	30
17	6,898,000	32,000	99.536	150	30
18	9,685,000	42,000	99.567	149	30
19	2,290,000	13,000	99.433	148	30
20	5,634,000	51,000	99.095	150	30
21	4,236,000	70,000	98.348	149	30
22	2,551,000	5,700	99.777	148	30
23	6,803,000	19,300	99.716	150	30
24	53,386,000	6,400	99.988	148	30
25	5,219,000	9,850	99 812	150	30
26	12,630,000	26,000	99.795	150	30
27		60,000		148	30
28	2,685,000	49,000	98.174	143	30
Average....	17,261,926	219,953	98.691		

ficiency of 98.69 per cent. In 17 cases the efficiency exceeded 99 per cent and in 4 cases more than 99.9 per cent. The fact that this work was done under practical conditions and with relatively

large volumes of mix may partly account for the comparatively lower pasteurization efficiency than has been obtained by Hammer and Sanders (12). By heating the mix to 145°F. and *not holding*, Hammer and Sanders were able to secure as high as 99.5 per cent reduction and an average destruction of 96.4 per cent of the organisms present. Tracy and Peterson (24) obtained better than 99.9 per cent reduction of bacteria by condensation of the mix. In the condensation process the mix is heated to 160° to 170°F. in the forewarmer, then condensed at 130°F. Bahlman (3) concluded from his work "that proper pasteurization and enforcement of regulations would reduce the bacterial content of ice cream, it would insure a safe product and give physicians some assurance of the safety of the product for invalids and children."

Examination of table 2 will show that there are 9 instances (experiments 1, 2, 3, 5, 6, 8, 11, 21 and 28), in which the efficiency of pasteurization is less than 99 per cent. In 6 of these cases (experiments 1, 3, 5, 6, 8 and 28) the temperature and time used for pasteurization did not comply with the laws of this state (Kansas) which require that all dairy products used in the manufacture of ice cream be pasteurized by heating to 145°F. for thirty minutes. In the other 3 of the 9 instances, (experiments 2, 11 and 21) the temperature and time used exceeded 145°F. for thirty minutes. However, if the data from experiments 2 and 11 be examined (table 1) it will be noted that the counts after pasteurization are apparently erroneous when compared to other counts obtained in the subsequent steps of the manufacturing process.

Further comparison of tables 1 and 2 will show that in six of the ten instances in which pasteurization did not meet the legal requirements, the bacterial content of the ice cream at the freezer was 100,000 per gram or greater. The data indicate very strikingly that the care of pasteurization is a primary factor in producing ice cream having a low bacterial count.

In all but two instances (experiments 15 and 16) in which 145°F. or a higher temperature for thirty minutes was used for pasteurization, the bacterial content of the finished product at

the freezer (column 5, table 1) was less then 100,000 per gram. Examination of table 1 will show that in these two exceptions the bacterial count of the mix after pasteurization was less than 100,000 per gram and that subsequent increase was apparently

TABLE 3

The total bacterial counts before and after homogenizing

EXPERIMENT NUMBER	MIX		INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
	Before homogenizing	After homogenizing				
1	170,000	233,000	63,000		37.0	
2	500,000	160,000		340,000		68.0
3	285,000	128,000		157,000		58.0
4	58,000	52,500		5,500		9.4
5	75,500	120,000	44,500		58.9	
6	2,210,000	2,830,000	620,000		28.0	
7	35,500	111,000	75,500		21.2	
8	124,000	132,000	8,000		6.4	
9	27,000	270,000	243,000		900.0	
10	70,000	109,000	39,000		55.7	
11	2,070,000	153,000		1,917,000		92.6
12	44,000	2,980,000	2,936,000		6672.7	
13	6,500	11,000	4,500		69.2	
14	20,000	20,000				
15	52,000	19,500		32,500		62.5
16	27,000	63,000	36,000		133.3	
17	32,000	36,000	4,000		12.5	
18	42,000	44,000	2,000		4.7	
19	13,000	16,000	3,000		23.0	
20	51,000	47,000		4,000		7.8
21	70,000	53,000		17,000		24.2
22	5,700	7,000	1,300		22.8	
23	19,300	27,600	8,300		43.0	
24	6,400	9,500	3,100		48.4	
25	9,850	12,200	2,350		23.8	
26	26,000	84,000	58,000		223.0	
27	60,000	12,000		48,000		80.0
28	49,000	29,000		20,000		40.8
Average.	219,953	277,475	57,520		26.1	

due to contamination. In experiment 15 the count is increased to above 100,000 per gram during the aging process, and in 16 the high count is apparently due to contamination from the

freezer. Therefore, these results, especially if considered in relation to the work of other investigators, indicate that if ice cream mix is pasteurized at 145° to 150°F. for thirty minutes, and the utensils do not offer a source of gross contamination, it is possible to manufacture ice cream under commercial conditions containing less than 100,000 bacteria per gram.

Homogenizing. Table 3 shows the effect of homogenization of the mix. There was an increase in the agar plate count 18 times and a decrease 10 times as a result of homogenizing. In general, however, the increases were larger than the decreases, and the averages of all the counts showed an increase of 26 per cent after homogenizing. Such an increase in bacterial count is no doubt more apparent than real, being due chiefly to the breaking up of clusters of organisms each individual of which may give rise to a separate colony on an agar plate. Hammer and Sanders (12) found that unless careful attention was paid to cleaning the homogenizer it would be a source of considerable contamination. It usually required from thirty to forty-five minutes to run the mix through the homogenizer, depending upon the quantity of mix that was made. Since homogenization was done at the temperature used for pasteurization, the last portion of the mix to go through the machine had been heated for an hour or more. This continued heating period made the efficiency of pasteurization even greater than is shown in table 2.

Aging. Table 4 shows the bacterial counts before and after the aging process. In 13 instances the number of organisms increased and in 15 cases there was a decrease. In half of the counts the increase or decrease is probably within the limits of experimental error.

The figures at the bottom of table 4 show that there was a difference of only 580 bacteria in the average counts before and after aging. This would indicate that in general the aging of the mix sixteen to twenty-four hours does not affect the bacterial count very markedly when the temperature is kept below 45°F. These results agree with those of Hammer (10) who found no increase in the count of the mix aged four days at

0°C. and also with those of Tracy and Peterson (24) who kept condensed mix for thirty-two days at 32° to 35°F. and found no marked increase in the number of bacteria until the fourteenth day.

TABLE 4

The total bacterial counts before and after aging

EXPERIMENT NUMBER	NUMBER OF HOURS AGED	BEFORE AGING	AFTER AGING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1	16	120,000	18,500		101,500		84.5
2	16	60,500	44,000		15,500		27.2
3	16	250,000	262,000	12,000		4.8	
4	16	29,500	35,500		4,000		10.1
5	16	79,000	79,500	500		0.6	
6	18	3,700,000	3,835,000	135,000		3.6	
7	16	95,000	244,000	149,000		156.8	
8	16	178,500	165,500		13,000		7.2
9	16	11,000	10,000		1,000		9.0
10	16	160,000	41,000		119,000		74.3
11	16	111,000	115,500	4,500		4.0	
12	16	23,000	27,000	4,000		17.3	
13	16	9,000	8,000		1,000		11.1
14	48	14,000	12,000		2,000		14.2
15	16	73,000	108,000	35,000		47.9	
16	15	152,000	70,000		82,000		53.9
17	24	16,000	63,000	47,000		293.7	
18	16	43,500	10,500		33,000		75.8
19	16	14,000	28,000	14,000		100.0	
20	16	54,000	48,000		6,000		11.1
21	24	73,000	33,000		40,000		54.7
22	16	5,200	9,800	4,600		88.4	
23	16	11,000	14,000	3,000		27.2	
24	16	8,200	6,800		1,900		23.1
25	16	11,400	8,400		3,000		26.3
26	16	25,400	16,300		9,100		35.8
27	16	7,700	8,350	6,500		8.4	
28	16	25,000	65,000	40,000		160.0	
Average..		191,782	192,362	580		0.3	

Freezing. The bacterial counts before and after freezing are shown in table 5. In order to eliminate the factor of contamination from the freezer, and to study the effect of the freezing operation on the bacterial count the samples were taken from the second and third freezing.

The data show an increase in the bacteria count as a result of freezing in 21 of the 27 samples with an average increase of 17 per cent. There were only six decreases, four of which (experiments 14, 19, 20 and 21) are probably within the limits of

TABLE 5

The bacterial counts before and after freezing

EXPERIMENT NUMBER	BEFORE FREEZING	AFTER FREEZING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1	18,500					
2	44,000	56,000	12,000		27.2	
3	262,000	188,000		74,000		28.2
4	35,500	52,500	17,000		47.8	
5	79,500	97,000	17,500		22.0	
6	3,835,000	2,880,000		820,000		22.1
7	244,000	1,165,000	921,000		377.4	
8	165,500	900,000	734,500		443.8	
9	10,000	24,000	14,000		140.0	
10	41,000	97,000	56,000		136.5	
11	115,500	160,000	44,500		38.5	
12	27,000	64,000	37,000		137.0	
13	8,000	20,000	12,000		150.0	
14	12,000	11,000		1,000		8.3
15	108,000	172,000	64,000		59.2	
16	70,000	150,000	80,000		114.2	
17	63,000	67,000	4,000		6.3	
18	10,500	26,000	15,500		147.6	
19	28,000	22,000		6,000		21.4
20	48,000	42,000		6,000		12.5
21	33,000	31,000		2,000		6.0
22	9,800	10,000	200		2.0	
23	14,000	29,500	15,500		110.7	
24	6,300	7,300	1,000		15.8	
25	8,400	11,650	3,250		38.6	
26	16,300	18,650	2,350		14.4	
27	8,350	12,000	3,650		43.7	
28	65,000	77,000	12,000		18.4	
Average.	192,362	236,688	44,326		23.0	

experimental error and are, therefore, insignificant. This concurs with the findings of Ellenberger (7) who states that, "aside from utensil contamination there is usually an increase in the number of bacteria resulting from the freezing process. This

is probably due to the breaking up of clumps of organisms." Hammer (13) agitated sterile water in a freezer and in five tests obtained counts as follows, 3700; 141,500; 8050; 1195; and 300 per cubic centimeter, thus illustrating the importance of the

TABLE 6

The influence of hardening on bacterial count of ice cream

EXPERIMENT NUMBER	BEFORE HARDENING	DAYS IN HARDENING ROOM	AFTER HARDENING	INCREASE	DECREASE	PER CENT INCREASE	PER CENT DECREASE
1							
2	56,000	2	67,500	11,500		20.5	
3	188,000	3	107,500		80,500		42.8
4	52,500	2	119,000	66,500		126.6	
5	97,000	2	102,500	5,500		5.6	
6	2,880,000	2	1,530,000		1,350,000		46.8
7	1,165,000	2	1,080,000		85,000		7.2
8	900,000	2	1,315,000	415,000		46.1	
9	24,000	2	30,000	6,000		25.0	
10	97,000	2	66,000		31,000		31.9
11	160,000	2	21,000		139,000		86.8
12	64,000	2	22,000		42,000		65.6
13	20,000	3	13,000		7,000		35.0
14	11,000	3	7,000		4,000		36.3
15	172,000	2	140,000		32,000		18.6
16	150,000	2	115,000		35,000		23.3
17	67,000	3	35,000		32,000		47.7
18	26,000	2	19,000		7,000		26.9
19	22,000	2	23,000	1,000		4.5	
20	42,000	2	34,500		7,500		17.8
21	31,000	2	30,000		1,000		3.2
22	10,000	2	7,400		2,600		26.0
23	29,500	3	11,800		17,700		60.0
24	7,300	2	30,500	23,200		317.8	
25	11,650	2	9,650		2,000		17.1
26	18,650	2	21,150	5,500		29.4	
27	12,000	2	8,150		3,850		32.0
28	77,000	2	62,000		15,000		19.4
Average..	236,688		186,320		50,368		21.2

freezer as a source of contamination. Hammer and Goss (11) found an increase in count after freezing in 84.3 per cent of their trials, the increase varying from 2 to 227 per cent.

Storage. Table 6 shows the difference in the total number of bacteria in the ice cream immediately after freezing and after forty-eight hours in the hardening room. There was an average decrease of 21 per cent.

The bacterial count decreased in 19 cases as a result of storage, and increased in 8 cases. It is improbable, however, that there was an actual increase in the number of organisms in any of these cases, due to the low temperature and short time of storage.

Stiles and Pennington (23) studied the changes in bacterial counts of four samples over a period of thirty-four days. In general the counts increased slightly between the first and third day, decreased gradually to the fourteenth day, then a marked increase occurred in all four samples on the twenty-seventh day followed by a sharp decline to below the original count on the thirtieth to thirty-fourth day. Esten and Mason (8) on the contrary were unable to find any such consistency in their study of 12 samples over a period of two months. Some of the samples increased and some decreased. They concluded, however, that there will be no marked increase or decrease in the bacterial content of ice cream stored for one month. Hammer (10) concluded that there was no tendency to increase the number of bacteria in ice cream on storage. Three of twelve samples which he examined showed a marked decrease in total numbers. Later Hammer and Goss (11) examined 39 samples of ice cream packed in salt and ice and 12 samples stored in a hardening room. Their findings indicate that during the proper storage of ice cream there is no increase in the number of organisms and there may be a decrease. The tendency to decrease apparently depends upon the bacterial flora of the ice cream. Ellenberger's (7) study of the changes in the bacterial content of ice cream in storage showed a slight decrease between the second and fourth day, after which there was a gradual decrease in viable bacteria. Ellenberger also showed that acid types (typically Bacterium lactis acidi) predominated throughout the period of storage.

This reduction in numbers may be due to the destruction of certain types of the less resistant varieties of bacteria present

in the ice cream. The low temperature of the hardening room would prevent any increase in such a short time as forty-eight hours. Therefore, the increases reported in table 6 are prob-

TABLE 7

Acid producers before and after pasteurization*

EXPERIMENT NUMBER	BEFORE PASTEURIZATION	AFTER PASTEURIZATION	DECREASE	PER CENT DECREASE
1	5,000,000	1,000,000	4,000,000	80.0
2	5,000,000	100,000	4,900,000	98.0
3	10,000,000	1,000,000	9,000,000	90.0
4	1,000,000	100,000	900,000	90.0
5	10,000,000	100,000	9,900,000	99.0
6	10,000,000	500,000	9,500,000	95.0
7	5,000,000	10,000	4,990,000	99.8
8	1,000,000	10,000	990,000	99.0
9	100,000,000	50,000	99,950,000	99.9
10	10,000,000	250,000	9,750,000	97.5
11	10,000,000	10,000	9,990,000	99.9
12	500,000	1,000	499,000	99.8
13	10,000,000	5,000	9,995,000	99.9
14	10,000,000	1,000	9,999,000	99.9
15	5,000,000	10,000	4,990,000	99.8
16	10,000,000	250,000	9,750,000	97.5
17	1,000,000	10,000	990,000	99.0
18	1,000,000	50,000	950,000	95.0
19	5,000,000	10,000	4,990,000	99.8
20	10,000,000	100,000	9,900,000	89.0
21	100,000,000	250,000	99,750,000	99.7
22	100,000,000	10,000	99,990,000	99.9
23	1,000,000	50,000	950,000	95.0
24	10,000,000	1,000	9,999,000	99.9
25	5,000,000	5,000	4,995,000	99.9
26	5,000,000	5,000	4,995,000	99.9
27	1,000,000	250,000	750,000	75.0
28	500,000	5,000	495,000	99.0
Average.....	15,785,714	147,964	15,637,750	99.0

* The term acid producer refers to the types that produce acid and no gas in lactose.

ably due in part at least to experimental error and not to an actual increase in the number present. The reduction in numbers varies with each different mix and is shown in table 6 to

range from 7 to 86 per cent. Such variation may be due to a difference in the number of the types present possessing low resistance to freezing temperatures.

TABLE 8

Acid and gas producers before and after pasteurization*

EXPERIMENT NUMBER	BEFORE PASTEURIZATION	AFTER PASTEURIZATION	DECREASE	PER CENT DECREASE
1	1,000,000	0	1,000,000	100.000
2	250,000	0	250,000	100.000
3	100,000	0	100,000	100 000
4	50,000	0	50,000	100.000
5	5,000,000	0	5,000,000	100.000
6	50,000	0	50,000	100.000
7	1,000,000	0	1,000,000	100.000
8	100,000	0	100,000	100.000
9	5,000,000	0	5,000,000	100.000
10	1,000,000	0	1,000,000	100.000
11	10,000,000	100	9,999,900	99.999
12	500,000	0	500,000	100.000
13	500,000	500	499,500	99.900
14	1,000,000	10	999,990	99.999
15	500,000	10	499,990	99.998
16	10,000,000	10	9,999,990	99.999
17	500,000	0	500,000	100.000
18	500,000	10	499,990	99.998
19	100,000	0	100,000	100.000
20	10,000,000	0	10,000,000	100.000
21	500,000	0	500,000	100.000
22	5,000,000	10	4,999,990	99.999
23	1,000,000	10	999,990	99.999
24	10,000,000	10	9,999,990	99.999
25	500,000	100	499,900	99.998
26	1,000,000	500	999,500	99.950
27	1,000,000	0	1,000,000	100.000
28	5,000	1,000	4,000	80.000
Average.....	2,362,678	81	2,362,597	99.996

* The term acid and gas producer refers to the types that produce acid and gas in lactose.

The effect of low temperatures on bacterial cells has been studied by MacFadyean and Rowlands (15), Park (17), Belli (4), Conn and Esten (6), Pennington (19), Ravenel, Hastings and

Hammer (20), and many others. Hammer (13) demonstrated that the low temperature at which ice cream is held can not be relied upon to destroy pathogenic organisms. Ice cream

TABLE 9

Gelatine liquefiers before and after pasteurizing

EXPERIMENT NUMBER	BEFORE PASTEURIZING	AFTER PASTEURIZING	INCREASE	DECREASE
1				
2				
3	500	500		
4	0	500	500	
5	0	1,000	1,000	
6	10	1,000	990	
7	100	500	400	
8	5,000	1,000		4,000
9	5,000	1,000		4,000
10	1,000	1,000		
11	0	100	100	
12	0	0		
13	0	500	500	
14	100	500	400	
15	0	100	100	
16	500	1,000	500	
17	100	100		
18	10,000	500		9,500
19	0	10	10	
20	100	1,000	990	
21	10,000	5,000		5,000
22	0	5,000	5,000	
23	0	100	100	
24	100,000	1,000		99,000
25	0	500	500	
26	100,000	5,000		95,000
27	0	5,000	5,000	
28	10	10		
Average.....	8,939	1,227		7,712*

* Average per cent decrease = 86.

that had been artificially inoculated with tubercular organisms was still infective for guinea pigs after one month of storage.

Types of bacteria in ice cream. The differentiation of the types of bacteria in ice cream was made on a basis of the action of the

organisms on lactose and on gelatin. Tables 7, 8 and 9 give the results of these differential counts on the samples before and after pasteurization. As previously described, the number of acid formers and the number of gas formers were determined by serial dilution of the sample in Durham fermentation tubes, containing lactose broth and brom-thymol-blue. The terms acid formers, and acid and gas formers, therefore, refer to the action of the organisms on lactose. The gelatin liquefiers were determined by serial dilution of the mix in nutrient gelatin. The highest dilution which failed to solidify in ice water after forty-eight hours incubation at 37°C. was taken as the number of liquefying types.

The number of acid forming types before and after pasteurization is shown in table 7. The average number of acid forming types before pasteurization was nearly 16,000,000 and only about 150,000 after pasteurization. There was no instance in which there were less than 1000 acid forming types per gram surviving pasteurization, thus illustrating the fact that pasteurization does not kill all of the acid producing types.

Pasteurization is very efficient, however, in destroying the gas producing types, as shown in table 8. In 16 of the experiments all of the gas producing types were killed and in seven instances there were only 10 gas producers per gram after pasteurization. The average number surviving was only 81 per gram. The number of these types in the raw mix varied from 5000 to 10,000,000 and averaged 2,362,000 per gram.

The gelatin liquefying types were not so consistently reduced by pasteurization. In 15 of the 26 experiments an increase was noted in the number of liquefying types after pasteurization. It will be observed in table 9, however, that the increases are relatively small and probably within the limit of the error of the method. Even though there may not have been an actual increase in the number of liquefying types, the data would indicate that in these 15 experiments at least, the number of gelatin liquefiers was not materially reduced. In 5 of the experiments there was a quite marked reduction in the gelatin liquefying types. These reductions were of sufficient magnitude

to make the averages of the 26 experiments show an 86 per cent decrease in the gelatin liquefying types after pasteurization. The average number of liquefiers was about 9000 per gram before, and 1200 after pasteurization.

Ayres and Johnson (1) made a qualitative examination of 71 samples of ice cream by the "milk tube" method. They found 30.84 per cent acid coagulating types, 38.03 per cent acid forming types, 5.42 per cent alkali forming types, 20.9 per cent peptonizing types, and 4.81 per cent of inert forms. Gas forming types were found in 0.1 cc. of 106 of the 120 samples examined.

SUMMARY

In this work an effort has been made to determine the possibilities of producing ice cream with a low bacterial content under commercial plant conditions. The conditions under which the work was done and the products used in some of the mixes were far from ideal. Care was taken not to employ methods to lower the bacterial count that were impractical or that could not be followed by any manufacturer.

Since the purpose of the work was to determine the possibilities of producing ice cream with a low bacterial count, a slightly higher temperature of pasteurization was employed in most of the experiments than is required by the state law. The data presented show that it is possible to produce ice cream consistently having less than 100,000 bacteria per gram, even though in many instances the raw mix contained several million per gram. It is also quite evident from the data that the bacterial count of the finished product is more dependent upon the temperature and time used in pasteurization than upon the original number of bacteria in the raw mix. In this respect the bacterial count of ice cream may not reveal the use of raw products of poor quality. It is possible for a manufacturer to utilize a high temperature of pasteurization to conceal the low grade of ingredients used in the mix. It is quite probable that some manufacturers would resort to such practices if bacterial counts were being determined on the ice cream. It would at least have the desirable effect of stimulating careful pasteurization.

One point repeatedly observed throughout the progress of the experiments was that any carelessness on the part of the ice cream maker invariably resulted in a high bacterial count of the finished product. If the pasteurization time or temperature were reduced, utensils not thoroughly cleaned, the temperature during aging was not properly controlled, a high count in the finished ice cream was almost certain to result. This observation was so consistent that it was even possible to predict a high or low bacterial count of the finished product from the time and care used in its manufacture.

The results in this paper on aging of the mix may not be applicable to those plants using a longer period of aging than was in use with this work. These results show practically no change in the bacterial count before and after aging except in a few instances. If these mixes had been aged three to six days, as is occasionally practiced, there would probably have been a slight increase in the number of organisms after aging. The temperature used for aging is such that bacterial growth is very slow and is negligible in fifteen to eighteen hours. When long aging periods are used, a slight increase may be expected, but if the temperature is properly controlled, this increase should not be sufficient to cause an excessively high count in the finished product. However, if the temperature is not well controlled and is allowed to raise only a few degrees the bacterial content will be greatly increased. Increasing the temperature ten degrees may mean an increase of several hundred per cent in the bacterial count after twenty-four hours. This increase in the bacterial content may be constituted of perfectly harmless types of bacteria, and the ice cream made from the mix may taste just as good as though the bacterial growth had been checked. It is the contention of the writers, however, that the failure to control the temperature during the aging process is indicative of careless methods and careless methods in turn indicate an undesirable if not an unsafe product. Hammer and Sanders (12) state that, "while in most ice cream of high bacterial content Bacterium lactis acidi, a harmless type, predominates, the entrance and rapid multiplication of this organism occurs under

conditions that make possible the entrance and multiplication of undesirable and possibly harmful types." Although it is true that ice cream having a high bacterial count due to a dirty plant, filthy utensils and poor raw products is much less desirable than a product having a high count due to growth of bacteria during the aging process, nevertheless it is difficult to determine to which factor the large number of bacteria is due.

This direct relation between the care of manufacture and the bacterial count indicates that a bacterial standard for ice cream would be particularly valuable. The increased use of ice cream emphasizes the necessity of controlling its manufacture. The consumer should be protected against the use of a product made under undesirable conditions. Since the bacterial count affords a fairly good check on the conditions surrounding the manufacture of ice cream, its use is recommended for such an index.

The problem of establishing standards is one that requires more data than is available at present. In this work ice cream has been consistently produced with less than 100,000 bacteria per gram by pasteurizing at 150°F. for thirty minutes. It remains to be shown what the bacterial count of ice cream should be when it is pasteurized at the minimum requirements of the present law, 145°F. for thirty minutes.

CONCLUSIONS

1. The temperature and time of pasteurization are the most important factors governing the bacterial count of ice cream.
2. A high bacterial content of the raw products used may be concealed by the use of high temperature of pasteurization.
3. The utensils need not be a source of gross contamination if they are properly washed and steamed.
4. Pasteurization at 150°F. for thirty minutes destroys practically all of the types of bacteria that produce acid and gas on lactose, but fails to destroy all of the types that produce only acid on lactose, or the types that liquefy gelatin.
5. Homogenization of the mix usually causes an increase in the bacterial count as determined by the agar plate method.

Such an increase is probably due to the breaking up of clumps of bacteria.

6. The aging of the mix for fifteen to eighteen hours at low temperature does not result in a material increase in the number of bacteria present.

7. As a result of the freezing process there is a slight increase in the agar plate count which is probably due to the breaking up of clumps of bacteria by the whipping action of the freezer.

8. There is a decrease in the number of bacteria during the first forty-eight hours of storage.

9. It is possible and practical to consistently produce ice cream containing less than 100,000 bacteria per gram by pasteurizing at 150°F. for thirty minutes and by using utensils only that have been thoroughly cleaned and steamed.

10. The bacterial content of ice cream serves as a good index to the conditions surrounding its production. Carelessness in handling the mix or cleaning the utensils will be revealed by a high bacterial count. Except in case of the use of a starter, ice cream containing large numbers of bacteria has been neglected at one or more steps in its manufacture, or it has been made from undesirable products.

11. The direct relation between the bacterial content of ice cream and the conditions under which it has been produced, suggests the value of establishing bacterial standards for this important food.

REFERENCES

(1) Ayers, S. H., and Johnson, W. T. Jr. 1915 A bacteriological study of retail ice cream. U. S. Dept. Agr. Bu. An. Ind., Bul. 303.

(2) Ayers, S. H., and Johnson, W. T., Jr. 1917 The determination of bacteria in ice cream. U. S. Dept. Agr. Bu. An. Ind., Bul. 563.

(3) Bahlman, C. 1914 Ice cream studies in Cincinnati. Amer. Jour. Public Health, iv, no. 11, 1009 to 1015.

(4) Belli, C. M. 1902 Der einfluss niederster, mit flüssiger luft erhaltener temperaturen auf die virulenz pathogenen keime. Centbl. f. Bakt., Abt. I, xxxi, no. 8, 355 to 360.

(5) Buchan, G. F. 1910 The contamination of ice cream. Jour. Hyg., x, no. 1, 93 to 130.

(6) Conn, H. W., and Esten, W. M. 1904 The effect of different temperatures in determining the species of bacteria which grow in milk. Annual Report of Storr's Experiment Station 1904, 27.

(7) ELLENBERGER, H. B. 1919 A study of bacteria in ice cream. Cornell Memoir 18.

(8) ESTEN, W. M., AND MASON, C. J. 1915 Bacterial content of ice cream. Storr's Experiment Station Bul. 83.

(9) FAY, A. C. 1923 The bacterial content of some Kansas ice cream. Jour. Dairy Sci., vi, no. 4, 283.

(10) HAMMER, B. W. 1917 Bacteria and ice cream. I. Iowa Agric. Exp. Sta. Bul. 134.

(11) HAMMER, B. W., AND GOSS, E. F. 1917 Bacteria in ice cream. II. Iowa. Agric. Exp. Sta., Bul. 174.

(12) HAMMER, B. W., AND SANDERS, L. R. 1919 Pasteurizing and homogenizing the ice cream mix. Iowa Agric. Exp. Sta. Bul. 186.

(13) HAMMER, B. W. 1921 The bacteriology of ice cream. Univ. of Wisconsin, Studies in Science, no. 2, 183–199.

(14) JORDON, JAMES O. 1922 Sense and sanitary control of ice cream. Ice Cream Trade Jour. xviii, no. 2, 53.

(15) MACFADYEAN, A., AND ROWLANDS, S. 1900 The influence of the temperature of liquid air on bacteria. Ref. E. S. R., xii, 913; Proc. Roy. Soc. (London) lxvi, 180.

(16) NEWMAN, G. 1899 Bacteria, p. 236. Putman and Sons, publishers, New York.

(17) PARK, W. H. 1901 Duration of life of the typhoid bacilli. Science, n. s. xiii, no. 322, 323.

(18) PENNINGTON, M. E., AND WALTER, G. 1907 The bacteriology of commercial ice cream. New York Med. Jour., lxxxvi, no. 22, 1013–1018.

(19) PENNINGTON, M. E. 1908 Bacterial changes in milk. Jour. Biol. Chem., iv, 353.

(20) RAVENEL, M. P., HASTINGS, E. G., AND HAMMER, B. W. 1910 The bacterial flora of milk held at low temperature. Jour. Inf. Dis., vii, 38.

(21) Standard methods for the bacteriological examination of milk. (1921) American Public Health Association, New York.

(22) State of Victoria 1906 Regulations as to ice cream. Government Gazette, November 14, 1906, 255–260; cited by Buchan, G. F. (see reference 5.)

(23) STILES, G. W., AND PENNINGTON, M. E. 1907 Bacteriological investigation of ice cream in the District of Columbia. Hygienic Laboratory Bul. 41, 249, Government Printing Office, Washington, D. C.

(24) TRACY, P. H., AND PETERSON, R. W. 1922 The condensation process in the manufacture of ice cream mix. Jour. Dairy Sci., v, no. 3, 273.

(25) WILEY, H. W. Hygienic Laboratory Bul. 41, 245, Government Printing Office, Washington, D. C.

(26) FABIAN, F. W., AND CROMLEY, R. H. 1923 The influence of manufacturing operations on the bacterial content of ice cream. Tech. Bul. 60, Michigan Agric. Exp. Sta.

THE RELATION BETWEEN YEASTS AND MOULDS AND THE KEEPING QUALITY OF BUTTER

DONALD BETHUNE SHUTT

Department of Bacteriology, Manitoba Agricultural College, Winnipeg, Manitoba, Canada

Received for publication April 12, 1924

Much work has been done in the past few years to demonstrate that pasteurized creamery butter is often highly contaminated with microörganisms during the making. Lund (1) and others have ably discussed this subject. Little has been accomplished, however, in relating the actual keeping quality of the butter, so contaminated, over a period of six months in cold storage. That there is a definite relation existing between this contamination and the keeping quality, there is no doubt, but published data is scarce.

During the season of 1921, it was my privilege to be employed in the Bacteriology Department of the Ontario Agricultural College, on the yeast and mould analysis of butter for the Ontario Butter Grading Station. The following are extracts from a thesis prepared at that time by the writer, and may prove of interest.

In all 573 samples of butter were examined during the season. They consisted of a plug, taken with a sterile tryer from a 14-pound box and placed in a sterile jar for shipment to the laboratory. The samples were sent a distance of fifty miles over night and were plated the following morning. The usual plating methods were followed with the exception that wort agar (acidified) was used. Twenty-one of these samples were selected during the early part of the season and held in cold storage at 10°F. They were scored from time to time as directed, by the Official Grader. More boxes would have been set aside, but unfortunately good samples were scarce.

Preparation of wort agar

	per cent
Wort, cleared and filtered	40
Water, distilled	60
Agar, market	1.5

The wort was ordinary wort used in the manufacture of beer and was obtained from the Springbank Brewery, Guelph. It is known as unhopped wort. It was cleared, filtered and sterilized in the laboratory. Before pouring the plates, the wort agar was acidified with 5 per cent sterile lactic acid solution: 10 cc. of the solution to 250 cc. of melted wort agar.

TABLE 1

The effect of contamination by yeasts and moulds on the original scoring of pasteurized creamery butter, and on the scoring after long storage at 10°F.

SAMPLE NUMBER	MONTH	DAY	YEAST COLONIES	OIDIUM COLONIES	PENICILLIUM COLONIES	SCORE				
						Original	August 18	October 10	November 29	December 20
1	6	18	*	0	0	38	38	37.5	36	36
2	6	11	*	0	0	40	39	39	38	38
3	6	20	*	1	0	41	39	39.5	39	39
4	6	17	*	0	0	40.5	40	40	39.5	39
5	6	13	*	0	0	40	38.5	39	39	39
6	6	11	*	0	0	39	39	38	36.5	36
7	6	24	*	0	0	38		38	38	37.5
8	6	25	*	0	0	38		38.5	37.5	36
9	5	28	*	1	0	38.5		38	37	36.5
10	5	27	70	0	1	39	39.5	39.5	39	39
11	6	2	20	0	0	39.5	39.5	39	39	39
12	6	6	80	0	0	39.5		39.5	39	39
13	5	16	130	0	0	39		38.5	38	38
14	5	14	20	0	0	39		39	39	38.5
15	6	1	*	1,000	0	39	39.5	39	37.5	37.5
16	6	28	*	32,000	0	37.5	38.5	38	37	36
17	5	25	2,300	1,200	0	38	38	38	36	37
18	6	20	*	150	0	40		38.5	38	38
19	6	27	120	180	0	39.5		39	38.5	38
20	6	20	*	600	0	41		39.5	37	37
21	5	6	35,000	0	350	38	38	37.5	37	37

* Over 100,000 colonies per cubic centimeter.

Flavor standards at the Ontario Butter Grading Station, 1921

Minimum for special grade	Flavor 41
Minimum for grade 1	Flavor 39
Minimum for grade 2	Flavor 37
Off grade, below	Flavor 37

The effect of contamination by yeasts and moulds on the original scoring and on the later scorings will be found in table 1.

DISCUSSION OF TABLE I

The effect of high yeast count

It will be noted that there are 9 samples containing over 100,000 yeast colonies per cubic centimeter, accompanied with fewer than two moulds. One sample is a special, 4 are in grade 1, and 4 are in grade 2 when first scored. There is quite a difference between these samples in the scoring. The creamery with the special, turned out that class of butter most of the season, as did some of the other four in grade 1. In accounting for this, it would appear that these creameries possessed a desirable type of yeast that gave the butter at scoring a delicate aroma. Such yeasts are known. Unfortunately, however, the scoring of December 20 shows a drop in all 9 samples; 4 to off-grade.

The effect of low yeast count

There are 5 samples having less than 200 yeast colonies per cubic centimeter, coupled with fewer than two moulds. They are all in grade 1 when first scored. After approximately six months in storage, 3 remain in grade 1, 1 has dropped half a point and the other has dropped 1 point. They have all held their flavor to a greater degree than the high yeast samples. The high yeast samples ranging from half a point to three points loss in flavor. The half point mentioned in the high yeast samples being in grade 2 when first scored. The manager of one of these creameries reported that he was able to place his June butter on the market in February, in grade 1, without any loss.

The effect of mould

There are 7 samples containing over 100 mould colonies per cubic centimeter. Four are associated with over 100,000 yeast colonies. One with 2300, one with 120 and the other with 35,000, nos. 17 and 21 are in grade 2 when first scored. No. 19 is in grade 1, but drops to grade 2. Of the 4 accompanied with over 100,000 yeasts, 3 are in grade 1 when first scored. This is probably due to a favorable yeast. The moulds would most probably be in the spore stage and would not have had time to

germinate and form mycelium. It will be observed, however, on glancing at the final scoring that all three have deteriorated, the greatest occurring with no. 20 which has dropped from special grade to grade 2.

SUMMARY

Twenty-one samples of pasteurized creamery butter were selected and held in cold storage at 10°F. for a period of approximately six months. It would appear that butter made from pasteurized cream and churned with the least possible contamination will hold its grade to better advantage, than butter made from pasteurized cream and later contaminated during the churning process.

ACKNOWLEDGMENT

Thanks are due to the Department of Bacteriology, of the Ontario Agricultural College, Guelph, for the full use of the laboratory, and to the Ontario Butter Grading Station, Toronto for their coöperation in regrading the above samples.

REFERENCES

(1) LUND, F. H. Yeasts in pasteurized cream butter and some suggestions as to their probable source. Abst. Bact. Baltimore, 1921, no. 1, 11.

ADULTERATED ACID AS A POSSIBLE SOURCE OF ERROR IN TESTING MILK BY THE BABCOCK METHOD

WM. E. PETERSEN

Division of Dairy Husbandry, University of Minnesota, University Farm, St. Paul, Minnesota

Received for publication February 16, 1924

I. INTRODUCTION

Commercial sulfuric acid as used in making the Babcock test varies greatly in appearance and strength depending upon age, impurities contained and other substances with which it may have come in contact. Acid takes on a decidedly dark color with age and usually weakens in its activity. The same is true with acid with which bits of cork or other organic substances has come in contact. It is therefore customary to consider acid, dark in color, to be weak and the tester uses a larger quantity than is prescribed in order to get satisfactory results.

Due to the impurities that it may contain, fresh sulfuric acid may have a pink or yellow tint. Sulfuric acid is also usually kept in earthenware jugs from which small quantities are poured as it is needed. Therefore an abnormal color does not attract as much attention as would be the case if the acid would be seen in larger quantities.

These facts increase the possibility of the user overlooking adulterants which may have been added to the sulfuric acid for the purpose of increasing the fat reading if such adulteration only slightly affect the color of the acid.

II. OBJECT OF THE INVESTIGATION

The author's attention was called to the possibility of adulterating sulfuric acid when a milk tester encountered acid to which oil of apparently a high grade had been added. Use of this acid resulted in tests so high that they could not be read in ordinary 8 per cent test bottles.

The object of this investigation was twofold: first, to determine if it is possible, by adding an adulterant to the acid used, to increase the fat percentage as shown by the Babcock test; and second, if it is found possible to influence the reading in this manner how may the adulteration of the acid be detected?

III. METHOD OF PROCEDURE

Sulfuric acid is used in testing milk for butterfat because it reacts rapidly with all the solids of the milk with the exception of the fat upon which it has no effect except when too concentrated. This, together with the fact that the substance added to the acid would have to be recovered, in part or in whole, in the fat column of the completed test in order to effect the reading, would make it seem reasonable that the substances suitable for this experiment would be limited to: (1) fats or oil substances, (2) fat solvents and (3) a combination of these two. In this investigation the study of substances added to sulfuric acid were divided into these three groups, hereafter referred to as group I, group II, and group III.

Under group I, fats or oil substances, the following were studied: butterfat, lard, cottonseed oil, linseed oil, olive oil, corn oil, cod liver oil, sperm oil and several of the mineral oils. Under group II, fat solvents, the following were studied: gasoline, benzine, ligroin, xylol, kerosene and ether. Under group III, combinations of the substances listed in groups I and II, each of the solvents in group II were studied when saturated with the fats of group I.

The first object was to determine whether or not the substances as listed in the three groups would increase the reading of the milk if added to the sulfuric acid used. In case the result was positive it was planned to study the effect upon the acid as to color, consistency and activity, and the effect upon the fat column of the completed test.

The next step was to determine, for those substances found to increase the fat reading, the relation between the amount used as an adulterant in the acid and the fat reading.

For each substance tested as a possible adulterant for acid, a series of six samples were prepared. Each consisted of 200 cc. of commercial sulfuric acid and the amount of the adulterant added was: 0.5, 1, 1.5, 2, 2.5 and 3 per cent. From each of these adulterated lots of acid, ten successive portions of 17.5 cc. each were added to samples from the same lot of milk measured into Babcock test bottles in the usual manner. The tests were completed according to the usual Babcock method including duplicates in which normal acid was used.

The third point of the investigation was the possibility of detecting such adulteration of the acid by observing the appearances of the acid when adulterated, the looks of the fat column of the completed test and by running blanks made by adding the amount of acid regularly used in the test to water instead of milk.

IV. DISCUSSION OF RESULTS

Group I

All the substances in group I, i.e., butterfat, lard, cottonseed oil, linseed oil, olive oil, corn oil, cod liver oil, sperm oil, and the mineral oils gave similar results. A 2 per cent addition of each of these substances in sulfuric acid gave a marked increase in the fat reading, varying from an increase of 0.65 per cent acid-lard mixture to 1.25 per cent acid-sperm oil mixture. Adulteration of the acid by any of this group can readily be detected from the appearance of the acid. The color of the acid changes quickly to a dark wine color or becomes black and of a sirupy consistency when any of these substances are added, even in very small amounts. All of these substances rise to the surface of the acid within a few minutes after mixing, floating about in black masses. Because of this fact, duplicate samples seldom check in testing. The fat column is usually dark but in some cases of good color.

Group II

All substances of this group, i.e., gasoline, benzine, ligroin, xylol, kerosene and ether behaved alike in that they all floated

on top of the acid, and with the exception of kerosene and ether, they behaved alike in all other respects.

Table 1 shows that a 2 per cent addition of each substance of group II to the sulfuric acid increased the fat reading of the milk where such adulterated acid was used, in some cases more than 2 per cent, except in the case of ether where the effect was negative. It will be noted that in no case did the first and the second test of the milk check where the adulterated acid was used. It will also be noted that in some cases the increase in the reading was greater than the per cent of substance added

TABLE 1

The effect of fat solvents as adulterants with sulfuric acid upon fat reading of milk where such acid is used in conduct of the Babcock test

ADULTERANT AND AMOUNT	FAT READING WITH ADULTERATED ACID		FAT READING WITH NORMAL ACID	
	First test	Second test	First test	Second test
	per cent	*per cent*	*per cent*	*per cent*
2 per cent gasoline	6.9	6.4	4.2	4.2
2 per cent benzine	8.0	7.9	6.4	6.4
2 per cent ligroin	5.0	4.7	3.2	3.2
2 per cent xylol	6.5	6.2	4.2	4.2
2 per cent kerosene	4.3	4.1	3.4	3.4
2 per cent ether	3.4	3.4	3.4	3.4

This table is typical of results secured in numerous trials. The size of the vessel in which the adulterated acid is kept and the rapidity with which the acid sample is poured are factors which make for a variation from the above.

to the acid. This is accounted for by the fact that all of these substances floated on the top of the acid and that in pouring off the acid into the acid measure more of the adulterant would be poured off in the first portion than in any of the subsequent ones.

Effect upon the acid. With the exception of kerosene, none of the substances of group II had any apparent effect upon the color or the consistency of the acid. Kerosene caused the acid to become of a greenish color that would arouse suspicion. The addition of any of these substances had no apparent effect upon the activity of the acid.

Effect upon the fat column. In addition to increasing the reading of the fat column, all of the substances of this group produced unusually clear fat columns. The fat column was of clear amber color, and in no case was a "burnt" fat column noticed.

Relation of the quantity of adulterant used to the increase in the fat reading. Of a large number of trials table 2 was selected as being typical of the results that were secured with the various

TABLE 2

Effect of gasoline as an adulterant of sulfuric acid

AMOUNT OF GASOLINE ADDED TO THE ACID	FAT READING OF SUCCESSIVE PORTIONS OF ADULTERATED ACID									
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.5	4.0	4.0	3.95	3.9	3.9	3.9	3.9	3.9	3.9	3.9
1.0	4.5	4.5	4.2	4.4	3.9	3.9	3.9	3.9	3.9	3.9
1.5	4.9	4.7	4.6	4.4	4.3	4.1	3.9	4.0	3.9	3.9
2.0	5.8	5.4	5.1	4.8	4.4	4.2	4.0	3.9	3.9	3.9
2.5	7.0	5.9	5.4	5.1	4.8	4.6	4.1	4.1	3.9	3.9
3.0	7.9	6.2	5.5	5.3	5.0	4.7	4.4	4.4	4.2	4.1
Normal acid	3.9	3.9								

Although duplicate trials did not check exactly, this table may be taken as typical results to be expected from additions of gasoline in varying amounts to sulfuric acid.

substances of this group. This table gives the fat reading secured by each of ten successive 17.5 cc. measures of gasoline-acid mixture. It will be seen that when the amount of gasoline added to the acid exceeds 2 per cent the first and second samples fail to check within the customary limit of 0.2 per cent. It will also be seen that with a 2 per cent addition of gasoline to the acid, each successive sample of adulterated acid poured off caused a decrease in the fat reading until the fifth or sixth portion when practically all of the gasoline had been poured off. In the case where the adulteration is 3 per cent of gasoline the fat reading was influenced up the tenth sample.

The apparent irregularity of the decrease in the fat reading with the different percentages of adulteration may be accounted

for by the difference in rapidity with which the acid was poured off into the acid measure. This would also account for the fact that in duplicate trials identical results were not secured. Results expressed in table 2, however, are fairly representative of results that may be expected.

Detecting the presence of group II. With the exception of kerosene none of the substances of this group can be detected readily by appearance. As they all float on top of the acid and are transparent, they are not noticed without careful examination unless present in relatively large quantities. The fact that if any of these substances are present in sufficient

TABLE 3

The effect of butterfat saturated solvents as adulterants

ADULTERANT	FAT READING OF SUCCESSIVE PORTIONS											
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Normal	Increase
2 per cent addition of saturated solution of butterfat in:												
Gasoline	6.4	6.4	6.4	6.3	6.4	6.4	6.4	6.4	6.4	6.4	5.2	1.2
Benzine	6.4	6.5	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	4.9	1.4
Xylol	4.4	4.4	4.4	4.5	4.4	4.4	4.4	4.4	4.5	4.4	3.7	0.7
Ligroin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	3.7	1.3
Ether	4.3	4.3	4.1	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	

Results are here shown of tests with ten successive portions of acid containing 2 per cent of a saturated solution of butterfat in each of the five solvents, gasoline, benzine, xylol, ligroin and ether. None of these additions effected the color of the acid, and with the exception of ether saturated with butterfat, all remained in a mixture with the acid for more than an hour after being thoroughly mixed.

quantities to influence any number of successive tests the first samples will not check within the permitted limits and will therefore arouse suspicion. Gasoline and benzine can be detected by odor and all of this group can be detected by running a blank test by the Babcock method where water is used in place of milk. These substances will then appear in the neck of the test bottle and the amount of addition can be determined.

Group III. Saturated solutions of fats in fat solvents

Saturated solutions of butterfat in either gasoline, benzine, ligroin, xylol or kerosene gave marked increases in the fat reading of milk when such saturated solutions were added to the acid used. A saturated solution of butterfat in ether increased the fat reading but slightly.

Effect upon the acid. Saturated solutions of these butterfat solvents effected the color of the acid very little when the acid was cool producing a very pale yellow. The coloration, however, was very slight. In a practical test an experienced milk tester preferred such adulterated acid to that of six months old acid which had turned dark in color. When these saturated solutions were added to warm acid the coloration was more marked. It was also noted that these saturated solutions remained in mixture with the acid for some time after being mixed. After standing for several hours they would rise to the top of the acid, but when agitated, would again go into a mixture with the acid. A saturated solution of butterfat in kerosene caused the acid to turn green. When ether and butterfat solution was added to the acid there seemed to be a reaction between the ether and the acid that liberated the butterfat which then floated on top of the acid in charred masses. Saturated solutions of the other fats and oils in these solvents caused a much more marked coloration of the acid.

The activity of the acid seemed not to be affected by the adulteration of any of these butterfat saturated solvents except that upon mixing milk and such adulterated acid, bubbles seemed to be formed immediately after the mixing was completed. This however, would not be noticed unless especially looked for.

Effect upon the fat column. Acid adulterated with saturated solutions of butterfat in gasoline, benzine, ligroin, kerosene or xylol produced unusually clear fat columns of amber color. The higher the percentage of adulteration the lighter in color was the fat column.

Uniformity of results. Table 3 was selected as expressing typical results found in numerous trials with saturated fat

solvents as adulterants. This table gives the results of each of ten successive measures of sulfuric acid containing 2 per cent of a saturated solution of butter fat in each of the various solvents. It will be seen that in the case of benzine, gasoline and xylol all ten tests checked within the customary limits. The slight variations may be accounted for by variations in the amounts of acid used, as the ordinary method of measuring acid is not accurate. In the case of ether and butterfat the successive samples did not check and after the third portion of adulterated acid there was no apparent effect upon the fat reading.

It will be noted from table 3 that the same percentage addition, to the acid, of saturated solutions of the different fat solvents did not give the same increase in fat reading. A 2 per cent addition of a saturated solution of fat in benzine gave an increased reading of 1.4 per cent while the same amount of a saturated solution of fat in gasoline and fat in xylol gave an increased reading of 1.2 and 0.7 per cent respectively.

Saturated solutions of other substances of group I in gasoline, benzine, xylol and ligroin increased the fat reading when added to the acid but so effected the color and consistency of the acid and the color of the fat column of the completed test that presence would be detected at once. For this reason detailed discussion of their behavior is omitted from here.

Detecting the presence in acid. Saturated solutions of butterfat in benzine, gasoline, and ligroin do not materially effect the appearance of the acid when added in moderate quantities. The fat columns of the completed test where such adulterated acid is used appears normal and duplicate samples of milk tested check within the permitted limits of variation. As a matter of fact there is nothing about the appearance of the acid or its behavior that will arouse suspicion. The only sure check being a blank test. A blank test on sulfuric acid containing 2 per cent of a saturated solution of butterfat in gasoline gave a reading of 1.1 per cent.

To get uniform results with successive samples of adulterated acid it was found necessary that the solvent must be saturated with butterfat. With gasoline it was found that if less than

15 grams of fat was added to 50 cc. of gasoline, part of the gasoline would rise to the top of the acid immediately after being shaken. In drawing off the acid, therefore, the first sample would contain a larger portion of gasoline than any of the succeeding samples and would give a larger fat reading.

It was also found that if oxidized butterfat was used in saturating the solvents there was less coloring of the acid.

V. SUMMARY AND CONCLUSIONS

1. Most fats and oils added to the sulfuric acid will increase the test of milk when such acid is used. All such fats and oils float on top of the acid and cause it to become very dark and sirupy, but do not effect its activity.

2. Many fat solvents when added to sulfuric acid will increase the test of milk where such acid is used. All float on top of the acid, but many do not effect the appearance of the acid nor its activity. Duplicate samples of milk where such acid is used will fail to check within permissible limits.

3. Saturated solutions of butterfat in gasoline, benzine and xylol will not materially affect the appearance of cold acid and will remain in solution with the acid for an hour or more after being thoroughly mixed. Successive samples of acid drawn will produce identical results; all samples checking in duplicate within the permissible limits of variation.

4. The presence of any substance in the sulfuric acid that will increase the test of milk when such acid is used in testing can be detected by making a "blank" test of the acid.

THE INFLUENCE OF PASTEURIZATION AND DIET OF THE COW ON THE ANTI-SCORBUTIC POTENCY OF THE MILK

THOMAS M. OLSON AND LYNN COPELAND

Dairy Husbandry Department, South Dakota State College, Brookings, South Dakota

Received for publication February 15, 1924

The vitamin content of milk apparently is influenced by two factors: viz., the diet of the cow, and the method or methods of preparing the milk for consumption. Kennedy and Dutcher (1) have reported that the presence of vitamins A and B in milk is entirely dependent upon the occurrence of these vitamins in the ration. This fact was substantiated by Osborne and Mendel (2) who observed no difference in the vitamin B content of milk produced on winter feed. Likewise Hughes, Fitch and Cave (3) fed cows a diet low in vitamins and observed that the A and B vitamin content of the milk to be very much decreased. The vitamin C content of milk appears to be more variable. Its presence in cows' milk is dependent upon the occurrence of the vitamin in the feed as well as the condition of the feed, and the manner in which it is cured.

Hart, et al. (4), of Wisconsin found that milk from cows which had been maintained on dried roughages and grains was lower in the anti-scorbutic vitamin than milk from cows on pasture. The following conclusions were deduced from this work:

Fifteen cubic centimeters dry feed-milk protected about six weeks.

Thirty cubic centimeters dry feed-milk protected about seven to eight weeks.

Fifty cubic centimeters dry feed-milk protected about twelve to fourteen weeks.

Seventy-five cubic centimeters of dry feed-milk gave complete protection over a period of eighteen weeks.

These data seemed to indicate that milk from dry-fed cows is deficient in vitamin C, but not wholly devoid of it. Hart

and Steenbock further concluded that the addition of silage and sugar mangels to the diet of the cow, did not greatly enhance the anti-scorbutic factor in milk. They report that the silage fed was made from corn well matured and partly dried before ensiling, a fact which might influence the anti-scorbutic value. The work of Hess, Dutcher, Kennedy and Unger seems to corroborate the work of Hart, et al., at the Wisconsin Station. All work on the various vitamins indicate that vitamin C is less stable in food products and is more easily destroyed by the various processes in the preparation of foods. This is no less true of milk, which is prepared for market.

There seems to be some differences of opinion among experimentalists as to the effects of pasteurization of milk in relation to its vitamin C content. Our work seems to point to the fact that there is a diminution of vitamin C, under all methods of pasteurization. The results obtained by Dutcher, et al., seem to indicate a greater destruction of vitamin C, when pasteurization is done in open vat or where oxidation can take place.

EXPERIMENTAL PROCEDURE

This experimental work was undertaken to determine the anti-scorbutic value of different amounts of milk when produced and treated under the following conditions: (*a*) Milk from cows on winter ration receiving corn silage compared with (*b*) milk from cows on winter ration, receiving no silage; (*c*) milk pasteurized in the bottle (final package) by the holding process 145°F., for thirty minutes, compared to fresh raw milk from the college herd.

The outline of purposes assumed that there might be differences in the anti-scorbutic properties of milk produced by cows on winter ration with and without silage. Also that the quality of the feed, including the condition and the method of curing are factors to be considered. The purposes further assumed that the methods used in pasteurization may also influence the vitamin content of milk.

Sixteen guinea-pigs were used for the work. Later 8 more were added. The pigs were young at the beginning of the trial

and weighed on an average of 300 grams. Before beginning the trial all the pigs were fed a complete diet. The pigs were weighed regularly, so as to detect any abnormal ones in the various lots. Each lot had about the same number of males and females and uniform in other respects. The pigs were kept in a cage with screen wire on both front and rear, each pig having a separate compartment about 12 inches square. Dry clean sawdust was used for bedding. The usual laboratory precautions were taken in regard to the sanitary conditions of the cages and dishes used for feeding. The milk was fed in aluminum dishes and were set in wooden blocks to prevent spilling and the pig allowed to drink milk ad libitum. The basal ration consisted of rolled oats, good quality autoclaved alfalfa hay cut fine, water, and salt. The alfalfa hay was autoclaved at 15 pounds pressure for thirty minutes and then dried.

Four pigs were placed on the basal ration; four more on basal ration plus pasteurized milk obtained from the College creamery. The milk was pasteurized in the bottle at 142° to 145°F. for thirty minutes and then cooled rapidly. Two pigs received 30 cc. daily, 1 pig 45 cc. daily and 1 pig 60 cc. daily of the pasteurized milk. In all cases the milk was fed in the dishes in two feeds, morning and evening. Four pigs were fed raw milk from the college herd plus the basal ration in the following amounts: 2 pigs received 30 cc., 1 pig received 45 cc., and 1 pig 60 cc. Later 4 more pigs were added to the experiment and fed the following amounts of milk from the college herd: 2 pigs received 15 cc. daily and 2 received 20 cc. daily plus the basal ration.

The college herd consists of the four principal dairy breeds with the Holsteins predominating. The herd was fed a grain ration of 4 parts of oats, 4 parts of corn, 2 parts of bran, and 1 of oil meal. During the early part of the winter and up to about February a very good quality of alfalfa hay was fed. The hay which was fed later in the year was not of as good quality. It was mixed with sweet clover, blue grass, and other tame grasses. The cows received all the corn silage they would eat. The silage was made from corn cut fairly green and was of very good quality.

Eight pigs were fed corresponding amounts of milk from a farmer's herd and the same basal ration. The herd from which this milk was obtained consisted of high grade Jerseys. Their grain ration consisted of ear corn and oats. For roughage they received an excellent quality of alfalfa hay up to the first part of February. This hay was very green and cured in cocks without caps. After the first week in February red clover hay was fed until April. From April until pasture season came on, a rather poor quality of alfalfa hay was fed. This hay was bleached and rather coarse also lacking in leaves. No succulent roughage of any kind was fed to the herd.

Composite samples of milk were taken over a period of several weeks and tested for butterfat.

Milk from college herd

	per cent
Composite sample 1	4.0
Composite sample 2	3.3
Composite sample 3	3.6
Composite sample 4	3.4
Composite sample 5	4.0
Composite sample 6	3.6
Average	3.65

Milk from farmer's herd

	per cent
Composite sample 1	5.1
Composite sample 2	5.4
Composite sample 3	5.5
Composite sample 4	5.0
Composite sample 5	5.1
Composite sample 6	5.3
Average	5.23

A complete analysis of the milk from the college herd was as follows:

	per cent
Total solids	12.61
Fat	3.7
Total protein	3.3
Lactose	5.0
Ash	0.74

The milk from the farmer's herd showed the following analysis:

	per cent
Total solids	14 2
Fat	5.3
Total protein	4.0
Lactose	4.2
Ash	0.75

The milk from both herds at all times appeared normal.

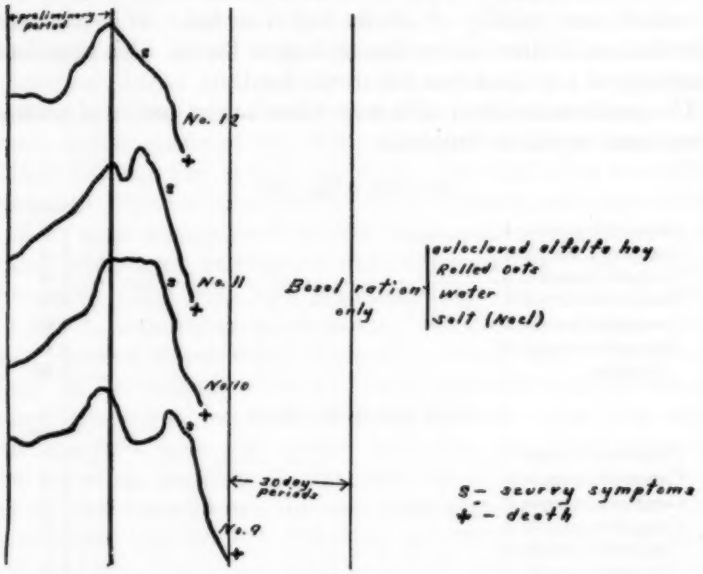


CHART 1

CARE AND MANAGEMENT

All animals were fed twice daily. A graduated pipette was used to measure the milk. All the milk was consumed before the following feeding period, except in several cases just prior to death. In no case was the milk force fed. The pigs were weighed every third day on a gram scale. All animals were autopsied as soon as possible after death. This work was done by a qualified veterinarian when the symptoms were not readily detected.

RESULTS

Chart 1 indicates clearly that the basal ration used was free from the anti-scorbutic vitamin. The rapid and uniform growth during the preliminary period is indicative of the good condition of the pigs before limiting their diet to the basal ration only. It is of interest to note also that all the pigs on the basal ration showed symptoms of scurvy about the same time, and 2 of the 4 died on the nineteenth day, the other 2 dying on the twenty-eighth and thirtieth days after being placed on the basal ration.

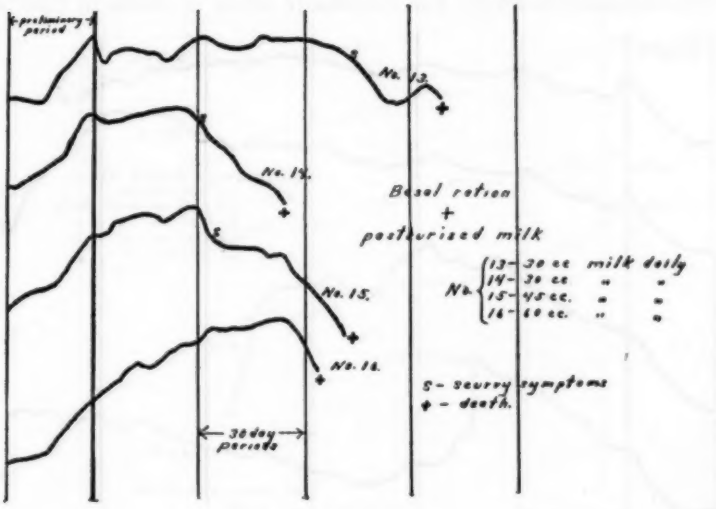


CHART 2

All the pigs were posted immediately after death, and the following conditions were noted: The costochondrel junctions were enlarged and inflamed. There were marked hemorrhagic areas in the thighs. The kidneys were also more or less hemorrhagic. Brittleness of the bones, particularly the leg bones was very noticeable. The contents of the cecum and intestines were soft and pasty; no cases of impaction was noted, on the contrary pig 9 had diarrhea for several days previous to death.

BASAL RATION AND PASTEURIZED MILK

The results of the trial with pasteurized milk in addition to the basal ration were variable. Pig 14 in chart 2 lived fifty-four days on 30 cc. of this milk daily, while pig 13 receiving the same amount of pasteurized milk did not die until the ninety-seventh day. Although making no gain in weight, pig 13 showed unusual resistance. Pig 15 receiving 45 cc. of pasteurized milk daily lived seventy-two days. Pig 16 on 60 cc. of pasteurized milk died on the sixty-fifth day. On post-mortem, scurvy symptoms were evident; however, the immediate cause of death in case of

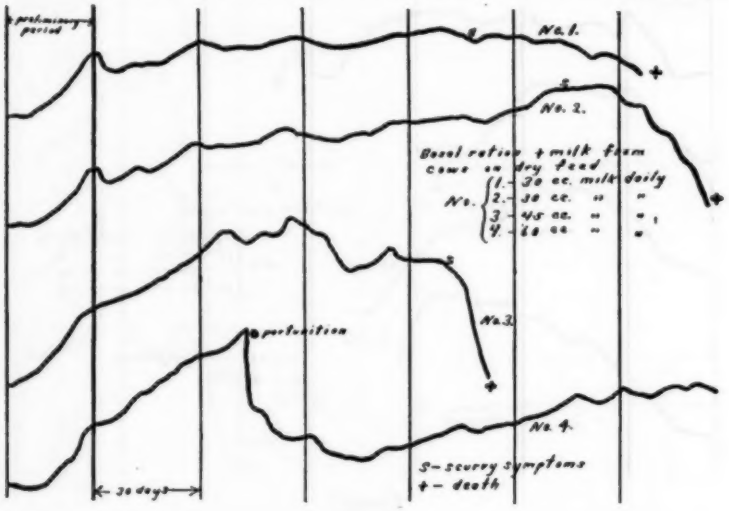


CHART 3

this pig was attributed to penumonia. It is possible that the lowered vitality due to the ration was a factor in bringing on pneumonia as this was the only case in which death could be attributed to any cause other than a deficiency ration.

BASAL RATION AND MILK FROM FARMER'S HERD

Pigs 1, 2, 3 and 4 on chart 3 were fed milk from cows on dry feed only for the duration of the experiment of twenty-five

weeks. Three of these pigs (nos. 1, 2 and 3) developed scurvy and died. Pig 3 died on the one hundred and eighth day. Death was probably hastened by loss of all four incisor teeth. The teeth became brittle and broke off at the gums. This made it difficult for the pig to eat solid foods. Pig 2 died a few days previous to the conclusion of the trial. Pig 4 was pregnant when the trial started, and after forty-two days on the restricted diet she gave birth to 1 young. The young pig showed no

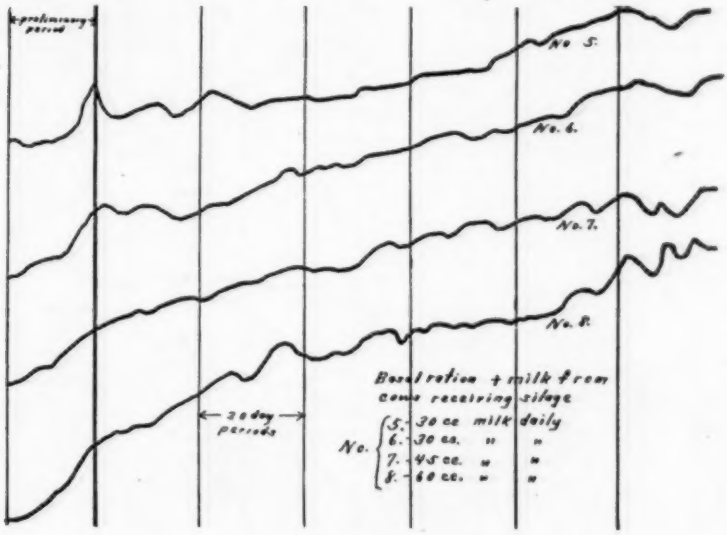


CHART 4

scurvy symptoms when born and was allowed to suckle for two weeks. Shortly after her parturition, pig 4 fell and injured her back. She recovered from these incidents and made good growth during the last sixty days of the trials.

BASAL RATION AND MILK FROM COLLEGE HERD

Chart 4 which shows the growth of pigs receiving milk from the college herd is a marked contrast to chart 3. The pigs shown on the latter chart received the same amount of milk

and the same basal ration. At the end of the trial all of these pigs were in a vigorous, healthy condition, with a glossy coat. Three of these pigs, no. 6 on 30 cc. of milk, no. 7 on 45 cc. of milk and no. 8 on 60 cc. of milk, doubled their initial weight during the trial.

Chart 5 shows the growth curve of two other lots of 4 pigs each, which were started on the trial February 28. Pigs 13′, 14′, 15′, 16′ received in addition to the basal ration the following

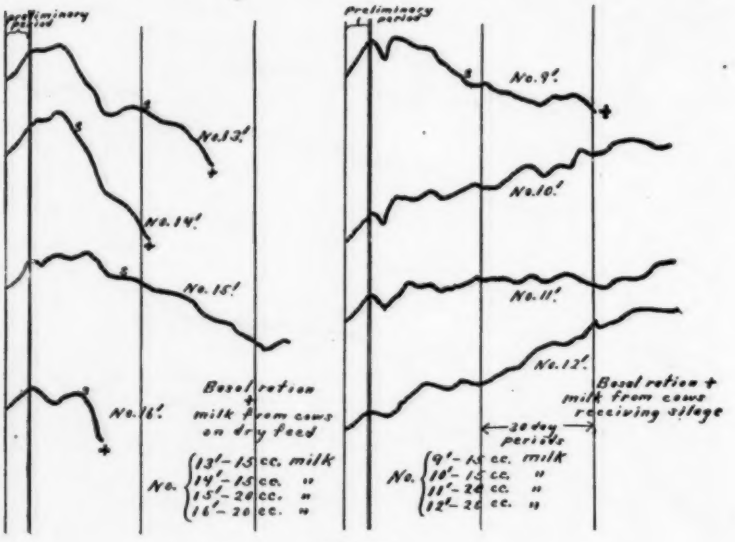


CHART 5

amounts of milk from the farmer's herd. Pigs 13′, and 14′, received 15 cc. of milk daily. Pigs 15′, and 16′, received 20 cc. of milk daily. All 4 of the pigs on milk from the farmer's herd developed marked symptoms of scurvy in less than thirty days. Two died within that time. Pig 13′ died on the forty-eighth day and pig 15′ did not die before the termination of the trial. Pigs 9′, 10′, 11′, 12′ were fed similar amounts of milk from the college herd. Pig 9′ was the only one which showed scurvy symptoms. (This pig died on the sixty-third day after the

trial began.) The other 3 pigs showed good growth, and their gains in weight were as good as could be expected on such small quantities of milk. The data in this write up comprises a period from November 4 to June 1 of the following year. The pigs which were still alive after this date were continued up to July 1 with results very similar to those already recorded. But inasmuch as both herds were then receiving more or less pasture which would involve another factor it was thought best to omit this data. It is of interest to record that no noticeable effect was observed on the pigs receiving milk from the farmer's herd, even after the herd had been on pasture for some time. However, the condition of the pigs at this stage of the trial no doubt was an important factor. A second trial involving the same factors was started last October. The results are essentially the same as those recorded herein. A more complete analysis of the three trials conducted at this institution on vitamin C in milk will appear in print later.

CONCLUSIONS

1. The results obtained from the pigs on the basal ration indicate that the ration used was free of vitamin C.

2. Pasteurization in closed bottles at 142°F., held at that temperature for 30 minutes diminishes the vitamin C content of milk.

3. These data indicate that the vitamin C present in milk has its origin in the feed.

4. Silage made from corn, cut when the kernels are glazed but before the lower leaves had dried increased considerably the anti-scorbutic potency of milk from cows receiving such silage.

5. Good silage contains sufficient vitamin C to maintain an adequate supply of this vitamin in the milk.

REFERENCES

(1) KENNEDY, C., AND DUTCHER, R. A.: The influence of the diet of the cow upon the quantity of the vitamins A and B in the milk. Jour. Biol. Chem., l, 339.

(2) OSBORNE AND MENDEL: Quoted by Funk: The Vitamines, 2nd edition, p. 257.

(3) HUGHES, J. S., FITCH, J. B., AND CAVE, H. W.: Scientific Proceedings, p. 50; Jour. Biol. Chem., xlvi,

(4) HART, E. B., STEENBOCK, H., ELLIS, N. R.: Influence of the diet on the anti-scorbutic potency of milk. Jour. Biol. Chem., xlii, 549.

(5) CHICK, HUME AND SKELTON: Anti-scorbutic value of cows' milk. Biol. Chem. Jour., 1918, xii, 131.

A COLORIMETRIC PICRIC ACID METHOD FOR DETERMINING LACTOSE

H. R. BIERMAN AND F. J. DOAN

Maryland Agricultural Experiment Station, College Park, Maryland

Received for publication April 28, 1924

In this paper we describe a picric acid colorimetric method for the determination of lactose in milk and some of its products. The results obtained by this method are compared with those obtained using the Official Gravimetric Fehling Method—Munson and Walker procedure.

REVIEW OF LITERATURE

The picric acid colorimetric method, for the estimation of carbohydrates, owes its origin to Dehn and Hartman (1) who worked with purified sugars and lactose in milk; and to Lewis and Benedict (2) who worked on glucose in blood. The method is based upon the fact that reducing sugars, in alkaline solution, (when heated) reduce picric acid which is yellow in color, to picramic acid which is a deep mahogany red. The original methods have been improved and modified for application to various sugar containing materials. Several different modifications, as applied to milk, have been published. Folin and Denis (3), report a modification using saturated picric acid and a standard which must have a color value within 20 per cent of the color value of the unknown. Pacini and Russell (4) use more complicated technique and solid picric acid with no restriction on the comparative colors of the standard and unknown. Boch (5), reports a study on the reaction of lactose on picric acid using Benedict's (6), "three times saturated solution," which is in reality sodium picrate. The method of Folin and Denis was used by Lisk (7) in her work on milk. To our knowledge, no one has applied any picric acid method to dairy products, other than milk.

EXPERIMENTAL

In the method described we have adopted the procedure of Folin and Denis for removing the protein and fat; but have used different dilutions and concentrations in the determination proper. A correction table has also been worked out to overcome the necessity of having more than one standard solution. Furthermore the method has been extended to cover some other dairy products.

In our preliminary work we found that pure lactose solutions and milk gave low results when they contained less lactose than the standard and high results when they contained more lactose than the standard, when calculated by the usual proportion method. This is due to the fact that the color developed is not proportional to the amount of lactose (or other sugars) present unless a much higher concentration of picric acid or sodium picrate is used (with greater complexity of technique) than under our procedure. It is for this reason that Folin and Denis require the colors of the unknown and the standard to the within 20 per cent of each other, thereby partially overcoming the error; and other modifications recommend sodium picrate (which is more soluble), solid picric acid, evaporation to dryness, etc., in their respective procedures. In the method reported here we have used a saturated solution of picric acid and overcome the error, otherwise present, by using a table of factors, which we have worked out. This table not only makes the procedure more simple but shortens the calculation of results somewhat.

The table of factors is based on 118 determinations made on 58 different lactose solutions. The difference in concentration of the lactose solutions varied from 300 to 700 mgm. in 500 cc. distilled water with 10 mgm. difference between individual solutions. This would be equivalent to from 3 to 7 per cent of lactose on the basis of milk. Color values representing these lactose solutions were obtained by the procedure described in this paper, using a 0.1 per cent water solution of lactose as the standard. The standard color was set at 20 on a Kober colori-

TABLE 1

Factors

A. Colorimeter reading of unknown compared with standard at 20.
B. Factor to use in calculation of results.

A	B	A	B	A	B	A	B	A	B
13.0	1.465	17.0	1.164	21.0	0.959	25.0	0.832	29.0	0.733
13.1	1.455	17.1	1.158	21.1	0.956	25.1	0.829	29.1	0.731
13.2	1.446	17.2	1.152	21.2	0.952	25.2	0.826	29.2	0.728
13.3	1.437	17.3	1.146	21.3	0.948	25.3	0.823	29.3	0.726
13.4	1.428	17.4	1.139	21.4	0.945	25.4	0.821	29.4	0.724
13.5	1.419	17.5	1.133	21.5	0.941	25.5	0.818	29.5	0.722
13.6	1.410	17.6	1.127	21.6	0.937	25.6	0.815	29.6	0.720
13.7	1.401	17.7	1.121	21.7	0.934	25.7	0.812	29.7	0.718
13.8	1.392	17.8	1.115	21.8	0.930	25.8	0.810	29.8	0.716
13.9	1.384	17.9	1.109	21.9	0.927	25.9	0.807	21.9	0.715
14.0	1.375	18.0	1.103	22.0	0.924	26.0	0.805	30.0	0.713
14.1	1.367	18.1	1.097	22.1	0.921	26.1	0.802	30.1	0.712
14.2	1.359	18.2	1.091	22.2	0.917	26.2	0.800	30.2	0.710
14.3	1.351	18.3	1.085	22.3	0.914	26.3	0.797	30.3	0.708
14.4	1.343	18.4	1.079	22.4	0.910	26.4	0.795	30.4	0.706
14.5	1.335	18.5	1.073	22.5	0.907	26.5	0.792	30.5	0.704
14.6	1.328	18.6	1.067	22.6	0.904	26.6	0.790	30.6	0.702
14.7	1.320	18.7	1.062	22.7	0.901	26.7	0.787	30.7	0.700
14.8	1.312	18.8	1.056	22.8	0.898	26.8	0.785	30.8	0.698
14.9	1.305	18.9	1.051	22.9	0.895	26.9	0.782	30.9	0.696
15 0	1 298	19.0	1.045	23.0	0.892	27.0	0.780	31.0	0.694
15.1	1.291	19.1	1.040	23.1	0.889	27.1	0.777	31.1	0.693
15.2	1.284	19.2	1.035	23.2	0.886	27.2	0.775	31.2	0.691
15.3	1.277	19.3	1.030	23.3	0.883	27.3	0.773	31.3	0.689
15.4	1.270	19.4	1.025	23.4	0.880	27.4	0.771	31.4	0.687
15.5	1.263	19 5	1.021	23.5	0.877	27.5	0.768	31.5	0.686
15.6	1.256	19.6	1.016	23.6	0.874	27.6	0.766	31.6	0.684
15.7	1.249	19.7	1.012	23.7	0.871	27.7	0.764	31.7	0.683
15.8	1.242	19.8	1.008	23.8	0.868	27.8	0.762	31.8	0.681
15.9	1.235	19.9	1.004	23.9	0 865	27.9	0.759	31.9	0.679
16.0	1.229	20.0	1.000	24.0	0.862	28.0	0.757	32.0	0.677
16.1	1.222	20.1	0.995	24.1	0.858	28.1	0.755	33.0	0.662
16.2	1.215	20.2	0.991	24.2	0.855	28.2	0.752	34.0	0.647
16.3	1.208	20.3	0.986	24.3	0.852	28.3	0.750	35.0	0.633
16.4	1.201	20.4	0.982	24.4	0.849	28.4	0.748	36.0	0.619
16.5	1.195	20 5	0.978	24.5	0.846	28.5	0.746	37.0	0.606
16.6	1.189	20.6	0.974	24.6	0.843	28.6	0.744	38.0	0.594
16.7	1.183	20.7	0.970	24.7	0.840	28.7	0.741	39.0	0.582
16.8	1.176	20.8	0.966	24.8	0.837	28.8	0.738	40.0	0.571
16.9	1.170	20.9	0.963	24.9	0.834	28.9	0.735		

meter. The lactose used was tested for purity by the Official Gravimetric Fehling Method (Procedure according to Munson and Walker) and a slight correction made before the standard was made up. A curve was then plotted—the abscissas being the concentration of lactose and the ordinates the colorimeter readings. It was then a simple matter to tabulate the lactose values of solutions corresponding to different colorimeter readings. This table constitutes an automatic correction and is a

TABLE 2

Recovery of lactose in solutions of known strength using table of factors

SOLUTION NUMBER	GRAMS WEIGHED OUT (PER 100 cc.)	GRAMS OBTAINED BY ANALYSIS (PER 100 cc.)	PER CENT ON BASIS OF MILK		VARIATION B-A
			Weighed out A	By analysis B	
1	0.0700	0.0696	3.50	3.48	−0.02
2	0.0842	0.0843	4.21	4.21	0.00
3	0.0932	0.0941	4.66	4.70	+0.04
4	0.1058	0.1050	5.29	5.25	−0.04
5	0.1219	0.1226	6.09	6.13	+0.04
6	0.1303	0.1366	6.91	6.83	−0.08
7	0.1331	0.1320	6.65	6.60	−0.05
8	0.1033	0.1008	5.11	5.04	−0.07
9	0.1117	0.1099	5.57	5.50	−0.07
10	0.1160	0.1164	5.80	5.82	+0.02
11	0.0659	0.0656	3.27	3.28	+0.01
12	0.0839	0.0844	4.19	4.22	+0.03

necessary adjunct to our procedure. Table 1 gives the factors with their corresponding colorimeter readings.

The accuracy of the table of factors was tested by determining the lactose in water solutions of known strength. Table 2 gives the results of this check. Pure lactose was weighed out, made up to 500 cc. and then recovered by analysis in terms of grams of lactose per 100 cc. and percentage on the basis of milk.

The results show that the accuracy of the method on the basis of milk is well within 0.1 per cent, the average difference being 0.04 per cent and the maximum difference noted on these solutions 0.08 per cent.

In our work thus far, several interesting and important ob-

servations were made. It was found that by filtering a portion of the solutions after the development of the color, higher colorimeter readings (lower colors) were obtained than on the unfiltered portions, showing that filtering results in a loss of color. By filtering the standard also it was possible to obtain the initial reading again. Because of this fact, if for any reason the unknown solution is filtered, after color development, the standard color solution must also be filtered to obtain accurate results.

Another point of importance, effecting results, unless procedure is accurately followed, is that after removing the solutions from the boiling water bath, the colors gradually fade for a period of time. For this reason it was found necessary to develop the standard color simultaneously with the unknown. This is also necessary because variations in length of heating periods, vigorousness of boiling, etc., give rise to variations in color. It therefore follows that a permanent color standard, except for approximate results, is not advisable; but if used, the time of heating and interval between heating and reading should be accurately controlled.

We attempted several methods of preserving the standard water solution of lactose for the use over a period of time. Formaldehyde gave a higher color when present in appreciable quantities and mercuric chloride on the other hand gave a lower color. It was therefore impossible to preserve the standard with either of these preservatives. We next tried making up the lactose solution in saturated picric acid. This method seems to give very good results. A standard was kept in saturated picric acid for over two months and the color developed at the end of that time was within experimental error of the color developed when freshly made. Either lactose is not inverted in appreciable quantities in a cold solution of picric acid, or what is more probable, the reducing powers of lactose, and the invert sugar from lactose, are the same on picric acid. We have made no thorough investigation of this point but some of our results would seem to indicate that even though inversion does take place, the final amount of picramic acid produced is the

same or so close that no difference is perceptible in the colorimeter. A standard solution in saturated picric acid was incubated at about 40°C. for five days, without any appreciable change in its final color value. We are, therefore, of the opinion that such a standard can be made up and kept for six or seven weeks for analytical purposes, without the necessity of preparing water solutions every day or so. This method of preparing a standard solution would be of value only where analyses are to be made daily or every few days, over a period of time. In using this type of standard solution it is advisable to use the same saturated solution of picric acid that is to be used in the subsequent determinations, since the amount of picric acid in solution should be the same and this is not always easy to be sure of.

METHOD AND PROCEDURE

Reagents

Picric acid. A saturated solution of picric acid in distilled water is used. Picric acid goes into solution extremely slow in the cold so a supersaturated solution is prepared by heating which is allowed to crystallize out at room temperature before use.

Sodium carbonate. Pure, anhydrous sodium carbonate is dissolved in distilled water at the rate of 22 grams per 100 cc. Heating facilitates the solution of the sodium carbonate.

Standard lactose solution. Exactly 1 gram of pure dry lactose monohydrate is dissolved in 1 liter of distilled water (or proportionate amounts in other volumes). For exacting work, a determination should be made on the lactose as a check on its purity, before making up the standard.

Note: As set forth in the description of experimental work, a standard may be made up in saturated picric acid and used for six or seven weeks with safety.

Preparation of standard color

Transfer 10 cc. of the standard lactose solution to a 100 cc. volumetric flask add 20 cc. of saturated picric acid (10 cc. when

the standard is made up in saturated picric, plus 10 cc. of water) and 10 cc. of 22 per cent sodium carbonate. Mix, cork lightly and heat simultaneously with unknowns in a boiling water bath for fifteen to twenty-five minutes; cool in cold water, simultaneously with unknowns, and dilute to 100 cc. Transfer a portion of the standard color solution to the colorimeter cup and place on left side of colorimeter, setting it at 20. The table of factors is based on the standard color being set at 20. (If set at any other point the reading of the unknown must be calculated to the value it would have if the standard was at 20, before the table of factors can be used.) The unknown solutions, representing the lactose content of the various samples, are compared with the standard color and readings taken. Due to the slight fading in color, it is advisable to change the standard color in the cup with each unknown and reset at 20. It is necessary to prepare a new standard color with each set of determinations and it is absolutely necessary to prepare the standard color along with the unknown under identical conditions for accurate results.

Milk

The specific gravity is determined with a Westphal balance or an accurate lactometer. With an accurate 2 cc. pipette, transfer 2 cc. of milk to a 100 cc. volumetric flask, previously half-filled with saturated picric acid solution. The pipette used should be one that will deliver exactly 2 cc. of milk. Fill to the mark with saturated picric acid, shake and filter. Transfer 10 cc. of the filtrate to another 100 cc. volumetric flask; add 10 cc. distilled water, 10 cc. saturated picric acid and 10 cc. of 22 per cent sodium carbonate. Mix, cork lightly and heat simultaneously with standard lactose solution, in a boiling water bath for fifteen to twenty-five minutes. Cool in cold water simultaneously with the standard color; dilute to 100 cc. and mix. Transfer a portion of the colored solution to the colorimeter cup and place in the right side of the colorimeter. The color is matched with the standard color in the usual manner. The average of five readings constitutes the color value.

Calculation. The percentage of lactose is found by obtaining the factor corresponding to the color value of the unknown solution from table 1 and using either of the following formulas.

$$\frac{\text{Factor} \times 10}{\text{Weight of sample}} = \text{per cent lactose}$$

or

$$\frac{\text{Factor} \times 5}{\text{Specific gravity of milk}} = \text{per cent lactose}$$

Cream

In determining the lactose content of cream, approximately 2 grams of cream are weighed into a 100 cc. volumetric flask previously half filled with saturated picric acid, using a Mojonnier weighing cross and pipettes. Fill to the mark with saturated picric acid, shake and filter. The subsequent procedure and calculation is then the same as for milk.

Whey

Approximately 2 grams or exactly 2 cc. of known specific gravity are used, with procedure similar to milk.

Evaporated milk

Approximately 1 gram of evaporated milk is weighed into a 100 cc. flask as for cream and the same subsequent procedure followed.

Powdered milk

In powdered milk we have a product which is often lumpy and it is advisable to mortar all samples before weighing out a representative portion. This applies especially to whole milk powders.

Weigh quickly, about 0.2 gram of the fine powder on a small watch glass. Transfer to a 100 cc. flask with the aid of a small funnel, washing the powder in with saturated picric acid. Shake vigorously before making to the mark or until no lumps of powder

are noticeable. Make up to the mark with saturated picric acid, mix and filter, proceeding as under aforementioned products.

Because of the small size of the sample and the large dilution. this method is not accurate to within less than 0.3 or 0.4 per cent of lactose in the powder. Other methods, however, have to contend with more or less the same difficulty depending on the amount of diluton used.

Ice cream

In ice cream we have a product, which not only contains lactose, but has considerable quantities of added sucrose as well. We have found by experimentation that the sucrose does not interfere with the determination of lactose by our procedure, providing that the sucrose does not become hydrolized during the time the product is in picric acid solution. Sucrose will invert comparatively quickly in the presence of saturated picric acid in the cold, and produce high results, whereas uninverted it gives no reduction.

In the determination approximately 1.8 grams of melted ice cream or ice cream mix, are weighed into a 100 cc. volumetric flask by means of a Mojonnier weighing cross and pipettes. The flask is then quickly made up to the mark with saturated picric acid, well shaken, and filtered. The first 10 cc. of clear filtrate are then rapidly transferred to another 100 cc. flask and the usual procedure followed. It is advisable to get the sodium carbonate into the flask within fifteen minutes of the time the sample is first introduced into the saturated picric acid, otherwise high results will be obtained due to inversion of some of the sucrose.

Condensed milk

Condensed milk has more sucrose present than does ice cream so that precautions in regard to inversion are even more important in analyzing it. The procedure is entirely the same as for ice cream except that only 1 gram of sample is used. It is

TABLE 3

Comparison of lactose content of various products by the colorimetric and gravimetric methods

PRODUCT	SAMPLE NUMBER	COLORIMETRIC METHOD (A)	FEHLINGS METHOD (B)	VARIATION A-B	COLORIMETRIC METHOD (1 cc.)
		per cent	*per cent*	*per cent*	*per cent*
Milk	1	4.75	4.82	−0.07	4.75
	2	4.70	4.76	−0.06	
	4	4.81	4.80	+0.01	4.75
	5	5.69	5.73	−0.04	5.74
	6	3.75	3.70	+0.05	3.81
	13	5.32	5.26	+0.06	5.31
	Average			0.05	
Cream	10	3.67	3.72	−0.05	3.68
	19	4.88	4.88	0.00	4.89
	20	3.71	3.72	−0.01	3.72
	Average			0.02	
Whey	16	4.65	4.65	0.00	4.70
	17	5.28	5.35	−0.07	5.22
	Average			0.04	
Evaporated milk	7	9.93	10.14	−0.21	
	9	7.86	7.72	+0.14	7.86
	18	9.59	9.45	+0.14	9.62
	24	9.34	9.30	+0.04	9.41
	Average			0.12	
Powdered milk	3	49.90	50.48	−0.58	50.05
	14	49.40	49.56	−0.16	49.75
	Average			0.37	
Ice cream	11	4.39	4.41	−0.02	
	12	3.87	3.96	−0.09	
	22	4.46	4.52	−0.06	4.45
	23	6.69	6.89	−0.20	6.64
	Average			0.09	
Condensed milk	8	10.08	9.80	+0.28	
	15	8.99	8.58	+0.41	
	21	12.35	12.45	−0.10	
	Average			0.26	

important to get a representative sample from the can or batch. Many cans have "sugar down" and representative portions are hard to obtain. In bad cases a primary dilution with warming, may be advisable. Such dilution should not be made with more than an equal volume of water, in which case 2 grams of the dilution may be used as the sample for analysis. It is advisable not to have any picric acid in the flask when the sample is weighed into it, since this will increase the time the sample will be in picric acid.

ACCURACY OF METHOD

In table 3 the results of several determinations by this picric method on the products listed under procedure are given, as are also the results on the same samples by the official gravimetric Fehling method. The amount of variation between results by the two methods is shown with the average for each product.

In the last column (headed "colorimetric method 1 cc.") we record results on some of the samples which were made up to 10 cc. instead of 100 cc. This was done by taking 1 cc. of the primary dilution (after filtering) instead of 10 cc. and using one-tenth the amount of reagents, the boiling being done in 10 cc. graduated test tubes and the tubes made up to 10 cc. just as the flasks are made up to 100 cc. The results by this variation in technique are fairly accurate and for many purposes this way of making the determination would doubtless suffice.

DISCUSSION

In view of the results obtained on the lactose content of milk and some of its products, the colorimetric picric acid method described, seems to be as accurate as the so-called "official methods," basing this assertion on a comparison with the Munson and Walker Fehling method. It has an advantage over the older methods in being quicker, less complicated, less tedious and in requiring fewer and more easily prepared reagents. The method is of special advantage where a large number of analyses are to be made at one time.

On fluid products, where a 2 gram sample is used, results are within experimental error, of the older method, averaging only about 0.04 per cent difference. On more concentrated products the variation in per cent is greater due to the greater dilution of sample that is necessary, or to the smaller sample taken. This however, is to be expected and most other methods have the same thing to contend with to a greater or lesser extent.

The main advantage of our modification over the method of Folin and Denis, we believe to be in the use of the table of factors which we have employed. This overcomes the error due to the fact that the color developed is not proportional to the amount of sugar present.

We expect to continue the work on this method and endeavor to apply it to determinations of sucrose as well as lactose in such products as ice cream and condensed milk.

REFERENCES

(1) Dehn, W. M., and Hartman, F. A. 1914 The picrate colorimetric method for the estimation of carbohydrates. Jour. Amer. Chem. Soc., xxxvi, 409.

(2) Lewis, R. C., and Benedict, S. R. 1915 A method for the estimation of sugar in small quantities of blood. Jour. Biol. Chem., xx, 61.

(3) Folin, O., and Denis, W. 1918 The determination of lactose in milk. Jour. Biol. Chem., xxxiii, 521.

(4) Pacini, A., and Russell, D. 1918 A method for the colorimetric determination of lactose in milk. Jour. Biol. Chem., xxxiv, 505.

(5) Boch, J. 1920 Notes on the colorimetric determination of lactose. Jour. Biol. Chem., xli; Scientific Proc., xiv.

(6) Benedict, S. R. 1918 A modification of the Lewis-Benedict method for the determination of sugar in the blood. Jour. Biol. Chem., xxxiv, 203.

(7) Lisk, H. 1924 A quantitative determination of the ammonia amino nitrogen, lactose, total acid, and volatile acid content of cows milk. Jour. Dairy Sci., vii., 79.

THE OFFICIAL TEST IN THE SOUTH AND THE PRELIMINARY MILKING[1]

EARLE BRINTNALL

Mississippi Agricultural Experiment Station, A. and M. College, Mississippi

Received for publication January 20, 1924

Over most of the South official testing was not undertaken as early as in the North. Some few of the Southern states, namely, Maryland, Kentucky and probably Virginia and Tennessee, did official testing from the first years. Since the arrival of the boll weevil the Southern farmer, who was formerly content with raising cotton, has taken up other lines of farming. A great many have become interested in dairying and this branch of farming has continued to attract attention at an increased degree with each succeeding year. Increased interest has created a larger demand for better cattle. This demand for improved cattle has induced an increased call for the Official Test each year. The growth of the Register of Merit of the Jersey Cattle Club in Mississippi well represents the advance of the Official Test in the South. Dairying was not followed we might say at all in Mississippi until 1909 or 1910 when the boll weevil commenced to make his work felt. In 1911 there were 3 cows entered in the Register of Merit from Mississippi; 1912, 3; 1913, 0; 1914, 0; 1915, 1; 1916, 3; and 1917, 4. In 1922, 36 cows were entered; an increase of 32 over 1917. The growth between these years was steady. This from one of the foremost cotton states well represents the growth of the dairy industry over a large section of the South. A large percentage of the breeders would be classed as small, and are testing 1, 2, or 3 cows at a time. It is a small man proposition.

Some of the things accomplished by the Official Test are:

1. Increased value of the animals completing the test.

[1] Read at the annual meeting of the Southern Division, American Dairy Science Association, Birmingham, Ala., January 10, 1924.

2. Increased value of the offspring of the animal tested. These two facts are substantiated by every sale where tested and untested animals are put up at auction.

3. An index as to the ability of the parents to produce animals with ability to produce and, in fact, to reproduce, or the value of the animals for breeding purposes.

4. Affects the mental attitude of the breeder himself, very many times because of his increased interest in his herd causing him to give it better care and feed.

The large breeder is better able to help himself than is the smaller breeder. He does not need outside aid and encouragement as does the smaller breeder. We believe that the small breeder is the one that should be encouraged to use the Official Test and to this end the test should be kept within his reach. The preliminary milking rule, if enforced will increase the cost of testing a cow on the average $3 to $4 a year. To the man with 1, 2, or 3 cows more will be added. Many breeders feel that they are paying the limit for testing now and to add an additional cost is the straw that will break the camel's back. Perhaps they are right. At least we should not add any additional expense until thoroughly proved necessary. Is the preliminary milking absolutely required to ensure a correct test? I wish to give some data collected at the Mississippi Station by the writer.

Our work is divided into five periods: normal, I, II, III, and IV.

Data collected in the spring of 1921 when tests were run on 8 cows for one month, during which the cows were under normal conditions and each milking was weighed and sampled and the sample tested for butterfat, was used to make up the Normal Period.

In the spring of 1923 we ran tests on the individual milkings of 2 Ayrshires, 2 Holsteins and 2 Jerseys for thirty days. These cows were in different stages of lactation, one advanced and one in the early stages being selected for each breed. They were milked twice a day. Three times during the thirty days milk averaging 36.6 per cent of the normal amount for that milking was left in the udder. This is known as period I.

Following this test, we ran similar tests on 5 Jerseys that were on Register of Merit work and were milked three times daily.

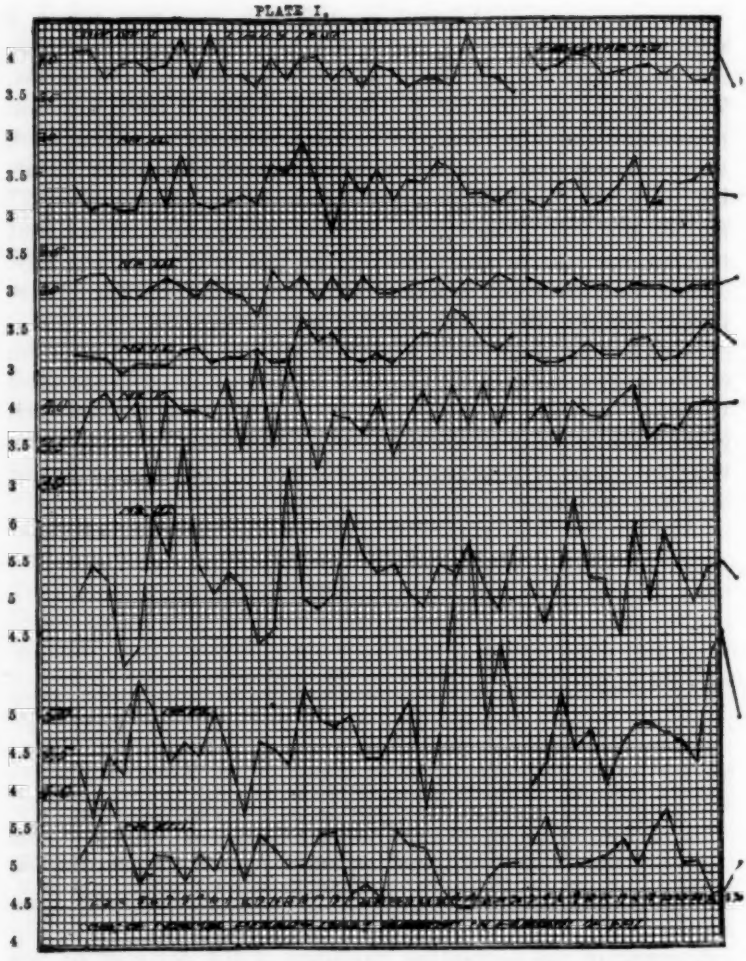
These cows were of different ages; 2 with their first calves, 2 with their third calves and 1 a cow over twelve years of age. All completed records that averaged over 500 pounds of fat. Two were in the eleventh and twelfth month of their lactation period and the other 3 in the third and the sixth. These cows were watched over a period of nearly seventy-five days. During the first two thirty-day periods, milk to the amount of 27 and 31 per cent, respectively, of the average amount for that milking was left in the udder at the last milking of the day. The last period of thirteen days was of normal milkings. These periods are periods II, III, and IV, respectively.

Does leaving the milk in a cow's udder influence the variation of the daily tests of her milk? In the following table the total variations of the tests of the milk of the individual cows of each group from one day to the next are collected and classified as to whether minus or plus and as to whether 0.4 per cent or less or over 0.8 per cent.

Daily variations of test

PERIOD	VARIATION												
	Total	0.4 per cent or less						Over 0.8 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	232	81	34.90	73	31.5	154	66.40	10	4.3	16	6.9	26	11.2
I	174	58	33.33	63	36.2	121	69.50	7	4.0	10	5.7	17	9.7
II	145	58	40.00	48	33.1	106	73,10	1	0.69	4	2.76	5	3.45
III	143	48	33.57	47	32.87	95	66.40	4	2.8	7	4.9	11	7.7
IV	48	21	43.75	11	22.9	32	66.65	2	4.2	0	0	2	4.2

Comparing the percentage variation of 0.4 per cent or less we find that the minus and the plus variations are about equal in all the periods. The greatest difference is in period IV when the cows were under normal conditions. The next greatest difference is in period II when the percentage of minus variations increases and this increase occurs at the expense of the



variations of greater than 0.8 per cent. The total variations of 0.4 per cent or less are more in the periods when the milking was interfered with, while the variations of more than 0.8 per cent are less. This study does not reveal any increased variation of the tests in the periods in which the milk was left in the udder.

We learn from this study that the tendency is for the amount of variation to increase as the average per cent of fat in the cow's milk increases and that the amount of variation varies with the individual cow. The test also seems to fluctuate from day to day about a certain medium point though this point may in the course of a month tend to either rise or decline. The plus deviations when totalled are always approximately equal to the sum of the minus deviations for that month. When there is a change in the test for a certain interval of time, usually one or two days, there is a corresponding change in the other direction, either following or preceding. This is very nicely brought out in the graph illustrating the variations of the test of the cows of the normal group as shown on figure 1. From this graph we learn that the least limit of variation is with the cow having the lowest average test while the greatest limit is with that cow having the highest average test. A study of the tests of the cows used during the other periods show that the limits of variation in the tests conform in a similar way to the average test and to the individual cow. This is illustrated on figures 2 and 3 with graphs showing the variations in test of the cows used in periods I and II.

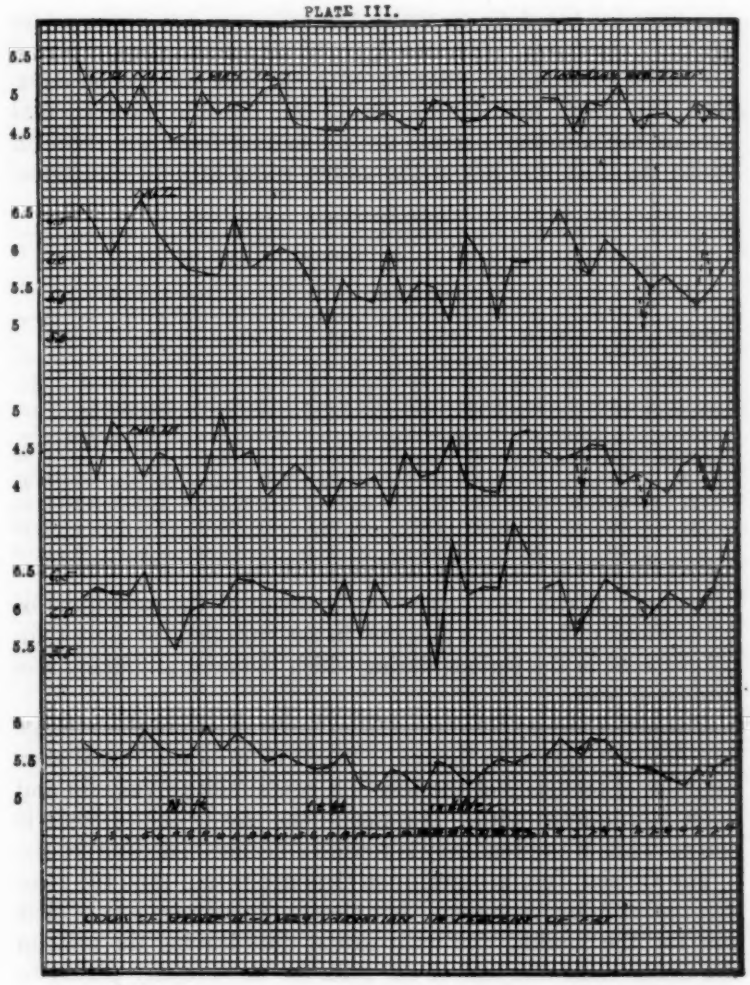
A study of the daily tests of the individual cows used in our work does not give any evidence that leaving the milk in the udder at any one time has influenced the direction of the variation of the test, unless on the day when the milk was left in the udder when the tendency is for the test to be lowered. Consider cow II (fig. 3). At the time the milk was left in the udder first her test shows a downward tendency. This downward slant continues the two days following the day on which the milk was left in the udder. At the next time the milk was left in the udder she also had a downward tendency. Leaving the milk in the udder threw the test lower and the following day it came back to exactly where it was and then fell again. At the last interference with the normal milking of this cow in this period she was due for a rise her test being very low the day before. This rise occurred on the day the milk was left in the udder her test going from 5.207 to 6.327 and then fell to 6.025. This

PLATE II.

decline continued the next day after which there was another rise in the test.

The Official Test is based on a two-day average test. Do the facts as given for the daily average hold for the two-day average?

PLATE III.



The following table shows the total variations of the average two-day tests of the cows of each group; the tests being collected and classified as to whether minus or plus and as to whether 0.3 per cent or less or over 0.7 per cent. In making this table

the tests for the days on which the milk was left in the udder are not considered. The two-day periods for the normal period are taken consecutively starting with the first and second days, then the third and fourth days, etc. In the experimental periods the two-day periods are all figured from the days on which the milk was left in the udder, going in both directions.

Two-day variations

PERIOD	Total	VARIATION											
		0.3 per cent or less						Over 0.7 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	112	31	27.7	47	42.0	78	69.7	5	4.5	5	4.5	10	8.94
I	72	25	34.7	27	37.5	52	72.2	3	4.2	2	2.8	5	6.95
II	60	25	41.7	19	31.7	44	73.3	1	1.7	1	1.7	2	3.34
III	59	22	37.3	22	37.3	44	74.6	0	0.0	1	1.7	1	1.70
IV	20	3	15.0	7	35.0	10	50.0	0	0.0	0	0.0	0	0.00

A study of this table shows an increase in the variations of 0.3 per cent or less and a decrease in the variations of more than 0.7 per cent in the periods during which the normal milking was interfered with.

A study of the graphs illustrating the variations of the average two-day test (given in figures 1, 2 and 3) does not reveal that the normal tendency of the test of a cow's milk to vary up and down is interfered with. If a rise in the test is due to come it comes, while if the tendency is toward a decline, a decline occurs.

The Official Test is based upon the supposition that the two-day test as taken represents very closely the average test of that cow for the month. To what extent does leaving the milk in the udder cause the test of the milk for the two-day period to vary from the average test for the month more than it normally would. The following table shows the total variations of the average two-days tests of the cows of each group from the average monthly test of each cow; grouping these tests as to whether 0.3 per cent or less or more than 0.5 per cent.

Variation of average two-day tests from average test for the month

PERIOD	Total	VARIATION											
		0.3 per cent or less						Over 0.5 per cent					
		Minus		Plus		All		Minus		Plus		All	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	120	49	40.8	45	37.5	94	78.3	4	3.3	7	5.8	11	9.16
I	84	31	36.9	35	41.7	66	78.6	3	3.6	6	7.1	9	10.7
II III	130	56	43.1	50	38.5	106	81.6	2	1.5	3	2.3	5	3.85
IV	28	10	35.7	10	35.7	20	71.4	1	3.6	1	3.6	2	7.14

In this table periods II and III are combined as they deal with the same cows. It will be noticed that a somewhat larger percentage of the variations in period I are over 0.5 per cent than is the case in the normal period. Three of these increased variations came before any milk was left in the udder at all and one increase came the fourth two-day period after leaving milk in the udder. There were three minus variations of over 0.5 per cent; one coming before any interference with the normal milking and one just before leaving the milk in the udder and one just after. We can thus account for a large number of the larger variations as taking place at a time when the milk left in the udder had no influence. On the whole the figures show decidedly less variation of the average two-day tests from the average monthly test in those periods in which the milking was not normal. Twenty-two of the average tests for the two-day periods after leaving the milk in the udder were greater than the average test for the month while twenty-six were less.

Throughout the whole trial the milk was left in the udder a total of 48 times. Twenty-eight times the average two-day test for the first two days after leaving the milk in the udder was greater than the test for the two-days before leaving the milk in the udder, and twenty times it was less. Seventeen times it was greater by less than 0.2 per cent and 12 times less by less than this amount.

Let us make further comparison of the average test for the two-day periods before and after leaving the milk in the udder. Adding the yield of milk and of fat for the first two days before leaving the milk in the udder of the first trial in periods I, II, and III and dividing to obtain the average test we have 4.779. Doing the same for the first two days after leaving the milk in the udder of the same trials we have 4.942. There is a difference of 0.163, greater after. Including the second and third trials of period II, which trials are separated from the previous trial by a period of eight days, we get a test of 4.950 before and one of 5.025 after, a difference of 0.075.

If we compare the third and fourth days before leaving the milk in the udder of the first trial of each period with the first and second days after we have 4.931 and 4.942, a difference of 0.011, greater after. Including the second and third trials of period II and using the third and fourth days before we get 5.047 before and 5.025 after, less after by 0.022.

The only change in the production of the cow that occurred consistently in this trial after leaving the milk in the udder was an increase in the flow of milk at the next milking. The cow always came back to normal on the second milking after. Nature has taken care of man's interference with her work and from then on the amount of milk secreted seems to be normal. On the average the increase in the amount of milk after will equal that amount left in the udder. The test of the milk for butterfat was sometimes more, sometimes less, and again evidenced no change at all. In our work no great advantage in test can be said to have resulted because of leaving the milk in the udder, and many times the test was lower. If milk was left in the udder we had ample proof in the amount obtained at the next milking compared with the other milkings of the two days following.

SUMMARY OF MINUTES OF MEETING OF SOUTHERN DIVISION, AMERICAN DAIRY SCIENCE ASSOCIATION, BIRMINGHAM, ALABAMA, JANUARY 10, 1924

J. A. GAMBLE

Maryland Agricultural Experiment Station, College Park, Maryland

Received for publication April, 1924

FRIDAY AFTERNOON, 2 P.M.

(a) Meeting opened by Professor Wylie, presiding.
President gave opening address.

(b) Moved that reading of minutes be dispensed with for the time. Also committee reports. *Carried.*

(c) Professor Borland stated that dairy industry exceeds the value of beef cattle plus twice hogs, plus twice sheep, in whole country.
Gave an outline of general instruction in more important institutions. He stated that the trend is to give all instruction in Dairy Husbandry Department.
One fourth of dairy cattle in U. S. is on Southern farms, also one sixth of the dairy products and one fifth of all milk.
He called attention to low farm butter prices in Southern States.
He also called attention to the low production of dairy cows in South. Called attention to need for college trained men in Dairy Manufacturing and surveyed the number of men taking dairying in colleges.

How Best to Fit or Train Men:

(1) Biggest factor is the teacher. Outlined the qualifications needed in such a man.

(2) Thorough training in fundamental subjects needed by students.

(3) Courses given will depend on number of students—facilities—instructors.

(4) Preservation of subject matter.
Best to use good texts and mimeographed notes.
Visual instruction should play large part—photographs, slides, charts, etc.
Proper dairy laboratories necessary.

(5) Summer practice should be required before graduation.
(6) Encourage investigation.
(7) Should keep in touch with graduates. Letter or start a round-robin.

(d) C. A. Hutton on Cow Test Association Work in South. (Paper submitted.)

Discussion on how to get testers—Suggested that more money be paid testers.

(e) Prof. J. A. McLean: Address on Feeding Dairy Cattle in South.
(1) Dairy barn and farm is a manufacturing plant and same principles apply as in any other plant.
(2) Four major factors—man, good cattle, good feeding, marketing.
The man should have greatest efficiency.
(3) Feeding is considered by speaker as most important factor of all.
(4) Ideal Feeding:
(1) Produce all feeds at home, particularly legumes and bulky feeds.
(2) Grow proper crops.
(5) Briefly outlined necessity of marketing by-products of milling processes.

(f) L. S. Edwards.

EVENING SESSION, 7:30 P.M.

(g) Paper on Improving Dairy Conditions in South, J. H. McClain.

(h) Report on Dairy Manufacturers Committee by L. S. Edwards. *Adopted.*

Mr. Thompson, Dairy Division, U. S. D. A. read an extract from a speech made by W. F. Jensen at American Association of Creamery Butter Manufacturers, at Chicago, December 4, 1923.

(i) Report of Committee on Nomination read and adopted.

(j) Motion made:

That the by-laws be amended to read that the election by marked ballot of the officers of the Section, be made within one month following the Annual Meeting. *Carried.*

Ayes: LaMaster, Thompson, Edwards, Borland, Thomas, Bennett, Arey, Baer, Hutton, Moore, Brintnall, McLain, Wylie, Holdaway.

(k) Paper read by Earl Brintnall. The Official Testing of Dairy Cattle. (Given elsewhere in this issue.)

(l) Report of Official Testing Committee, Professor Moore, read and adopted.

(m) Report of Extension Committee, C. A. Hutton. *Adopted.*

(n) Report Committee on Instruction in Dairying, J. A. LaMaster. *Adopted.*

(c) Dairy Production Committee, Stanley Combs reported.

(p) Judging Contest Committee.

Names of those present

John Aigdon, C. of Ga.....Columbus, Georgia
J. S. Moore, A. & M. College.....Mississippi
Eugene Baker, A. & W. P. RR.....Atlanta, Georgia
H. L. Alsabrock, A. B. & A. Ry.....LaGrange, Georgia
S. W. Heath, Dist. Agent.....Gainesville, Florida
H. G. Clayton, Dist. Agent.....Gainesville, Florida
J. A. Evans, Asst. Chief Officer.....Extension Work
Hamelin L. Brown, Dairy Ext. Agt.....Gainesville, Fla.
J. P. LaMaster,.....Clemson College, S. C.
L. S. Edwards.....Lebanon, Tenn.
C. A. Hutton.....Knoxville, Tenn.
J. A. McLean.....Chicago, Ill.
J. A. Arey, Dairy Ext.....Raleigh, N. C.
D. H. Upshaw, Ga. RR.....Atlanta, Ga.
A. C. Baer, Oklahoma A. & M. College.....Stillwater, Okla.
W. H. Eaton.....Auburn, Ala.
Earl Brintnall, A. & M. College.....Mississippi
G. W. Humphrey, Sou. Ry.....Atlanta, Ga.
H. S. McLeredon, F. E. C. Ry.....St. Augustine, Fla.
G. E. McWhorter, Agri. Agt. C. of Ga. Ry. Co.....Milledgeville, Ga.
S. C. Thompson, Dairy Division.....Washington, D. C.
J. L. Thomas.....College Station, Texas
Frederick W. Bennett, Ga. State College Agri.....Athens, Ga.
J. H. McClain, Dairy Division, U. S. D. A.....Washington, D. C.
Mrs. J. K. McDowell, Home Economics Dept.....Soft Wheat Millers, Assoc., Nashville, Tenn.
J. K. McDowell.....Feed Dept. Ballard & Ballard Co., Louisville, Ky.
Dr. Tait Butler.....Progressive Farmer
C. E. Wylie.....Knoxville, Tenn.
C. W. Holdaway.....Blacksburg, Va.
A. A. Borland.....State College, Pa.
Stanley Combs.....N. C.

RESOLUTIONS OF THE DAIRY SECTION TO ASSOCIATIONS OF AGRICULTURE WORKERS

Resolved: That this Association go on record as favoring the redoubling of the efforts of all interests toward completing the eradication of ticks which at present are retarding and preventing the development of dairying in large areas of the Cotton Belt.

Further resolved: That every effort be put forth to encourage and advance the eradication of tuberculosis and the control of contagious abortion in dairy herds.

Further resolved: That the efforts for developing the creameries in the South be especially directed along quality lines.

Further resolved: That it is more important to increase the production of the present cow population in the South by better feeding than to increase the number of cows or dairy farmers, to this end, i.e. urge the agronomist and county agent forces to put forth special effort to get produced an adequate supply of home grown feeds including pastures.

J. A. GAMBLE,
Secretary and Treasurer.

REVIEW OF FOREIGN DAIRY LITERATURE

H. A. BENDIXEN

University of Idaho, Moscow, Idaho

DRUGE, F. Two Short Researches on Milk. Lait, vol. 12, 1922, p. 101–103.

1. The effect of chloroform and toluol on rennet coagulation: Fresh morning's milk was placed into sterile Erlenmeyer flasks. To one sample was added chloroform at the rate of 14 grains per liter, to the second toluol at the same rate and the third was left without any addition. Each sample then received 1 mgm. of rennet per 100 cc. of milk and was held at a temperature of 34 to 35°. Complete firm coagulation took place in 122, 92 and 87 minutes respectively. Chloroform therefore inhibited rennet coagulation more than toluol.

2. The effect of chloroform and toluol on the spontaneous coagulation of cows' milk: One row of samples remained at 18°, the other at 5°. Chloroform plainly inhibited the action of the lactic acid bacteria; toluol less so. Milk may be kept five days by the addition of chloroform and the application of cold without increasing the acidity.—Matouschek (Vienna).

CATFOLIS, EM. Les présures microbiennes. Compt. Rend. Soc. Biol., Paris, vol. 87, 1922, p. 381–383.

Bacteria coagulate milk with the production of lactic acid following the production of a rennet like enzyme which is also secreted in casein free media. The enzyme production is a normal function of the cell. This bacterial enzyme produces anti-bodies in animals and the bacterial rennet enzymes are different among their kind and also different from the animal rennet enzymes. Differentiation is possible by means of the anti-serum.—Matouschek (Vienna).

RAHN, OTTO. The Importance of Surface Tension Facts in the Dairy Industry. Kolloidzeitschrift, 30, 1922, p. 341–346.

Special experiments to force one to conclude the existence of a film substance in milk not as yet demonstrated in pure form, but which presumably is of protein nature and which may at once explain the formation of milk foam, whipped cream, butter, the skin on heated

milk and the light scorching of milk. The fat in the butter forms no continuous structureless phase. During the initial drying up of the milk the film surrounding the fat globules which consists of the formed film substance is directly visible under the microscope.—Matouschek (Vienna).

WILLE, JOHANNES. Biological and Physiological Observations and Experiments on the Cheese Fly Larva; Piophila casei L. Zool. Jahrb. Abt. f. Allgem. Zool. n. Physiol. d. Tiere., Bd. 39, 1922, S. 301–320, m. 4, Textabb.

In combatting animal pests it is of great scientific interest, to determine which organs a combat material attacks and what changes they experience. Naturally therefore a thorough investigation of the biology and physiology of each pest must precede. With a gassy combat substance the respiratory organs are most important and besides, for example, the diffusion through the skin and absorption by the blood or the body cavity fluid. Here naturally the physical conditions of the gases and their chemical composition are most important for the action upon the animal body as well as the consideration of injury to colors, fabrics, metals, food materials, etc. when using gas. Besides the knowledge of the normal life processes of the pests is necessary to examine into the pathological conditions and irritations by the gases.

If for instance gas is allowed to act upon the cheese fly larva which is so detrimental to cheese, quite definite effects result on the larvae which are manifested by restless crawling around, jumping, etc. The task of the author was therefore to observe the several stimulations on the Piophila casei and to draw conclusions from these as to the biology and physiology of the insect.

The results of his studies were:

1. The crawling of the cheese fly larva is an alternating push and pull movement.

2. The forward movement by jumping is not found during the larva stage I and II, but only in the larva stage III and then most frequently and plainly with larvae which will pupate in a day.

3. The larvae III show negative phototropism.

4. As morphologically important parts of the larva body for the skipping process must be considered the head and posterior parts, which show special differentiations. These special parts are of greatest importance for the details of the skipping process.

5. The skipping process consists of bending the body together,

anchoring both body ends, stretching, and snapping off. The motion in skipping is in the direction of the head. The essentials for the jump are the muscle tensions in the larva body, the longitudinal muscles exerting a pull and the ring-muscles a pressure. The effect of the jump is increased by the initial stoppage of movement. A glueing function by means of special secretions does not exist.

6. As stimulations for the skipping process were experimentally determined mainly the influence of the light, in second place the influence of moisture. Temperature stimulations, air currents, mechanical and chemical stimulations are of no significance for the skipping.

KICKINGER, H. The Decomposition of the Citric Acid of Cows' Milk by Some Bacteria. Biochem. Ztschr., Bd. 132, 1922, S. 210.

Comparing the experimental results ascertained by the author the following conclusions seem justified.

1. The citric acid content in pasteurized and boiled cows milk is the same when determined immediately after heating the milk as when the milk is fresh; however, it decreases more or less on prolonged standing. In fractionally sterilized cows' milk as compared with fresh milk the citric acid shows a rather marked decrease during the first days. After the third sterilization, however, the citric acid content remains constant.

2. The cause of the decrease of the citric acid is no doubt found in bacteria and in the experiments under consideration they were representatives of the group of peptonizing bacteria (Bac. subtilis) Bac. mesentericus vulgatus and Proteus vulgaris), while lactic acid formers (3 different species, as well as a species of Joghurt bacilli) had no effect on the citric acid of the cows' milk.

3. The enantimorphic action of some lactic acid formers on one side and the peptonizing bacteria on the other allows with only slight probability of accuracy to draw conclusions as to the kind of bacteria from the amount of the citric acid present.—Heuss (Berlin).

FROG, F., AND SCHMIDT-NIELSEN, S. The Fatty Acid Content of Butterfat. Biochem. Ztschr., Bd. 127, 1922, S. 168.

The fatty acids of the examined butterfats were as follows:

	per cent
Butyric acid	3.4
Caproic acid	3.3
Capryllic acid	1.9
Capric acid	3.0

Lauric acid	3.7
Myristic acid	12.9
Palmitic acid	20.8
Stearic acid	6.2
Oleic acid (maximum values)	27.0
Not definitely identified acids (Gadoleic acid?)	
Linoleic acid?, acid C_{20} H_{30} O_2? arachic acid	
Behenic acid?	9.8
Distillation remnants	8.0

—Heuss (Berlin).

BLEYER, B., AND SEIDL, R. Contributions to the Knowledge of Cows' Milk Casein. Biochem. Ztschr., vol. 128, 1922, p. 48–75.

In fresh milk the casein which is almost insoluble in water is combined with CaO as "Söldners Casein-lime Compound" which is very strongly dispersible in water and which forms with it colloidal solutions, salt solutions. To separate the casein from the milk there are two methods available:

Forcing the casein out of its natural state by means of acidulation and by precipitating by means of the rennet enzyme. The laboratory experiments showed in regard to composition only a slight difference between acid- and enzyme casein; under the conditions at hand the former proved much more active than the latter. There is something conflicting. The authors first sought a casein as nearly as possible free from ash by a carefully described method. The N-factor of the acid casein is 100:15.5 equal 6.45, that of the paracasein 100:15.64 equal 6.39. Each gram of these two substances required 8.74 cc. of base for neutralization with phenolphthalein, which gives an acid equivalent of 11.45. Both caseins react with the alkaline earths according to the law of Henry, but they are not true compounds. The acid casein and paracaseins respectively result from adsorption, and the greatest adsorptive ability occurs with HCl. The paracasein can take up more acid than the casein.—Matouschek (Vienna).

DAIRY NOTES

Dairy Division, B. A. I. United States Department of Agriculture

Jackson, H. C., a graduate of Cornell University, and with a Ph.D. degree from that institution, has been appointed for research work in the manufacture of dairy products and by-products. Mr. Jackson has served as assistant professor in dairy manufacture at Cornell University, and has had practical work in plants manufacturing powdered milk, condensed milk, butter, cheese and market milk.

Frazier, William C., a graduate of the University of Wisconsin, and with a Ph.D. degree from that institution, has been appointed for research work in the bacteriology of milk. Mr. Frazier has served four and one-half years as instructor in the University of Wisconsin. For two years he was with the American Expeditionary Force, a part of the time working on the diagnosis of various diseases at the Central Medical Laboratory, Dijon, France.

THE REVIVAL OF DAIRY RESEARCH IN GERMANY

Agricultural research, particularly dairy research, revived vigorously in Germany during the past year. The first attempt after the war to reëstablish this important work failed. This condition was attributed to the breakdown of the German finances. Forschungen auf dem Gebiet der Milchwirtschaft, a scientific dairy journal was published for a period of eighteen months during this depressing time and discontinued.

At present the German government maintains a pure dairy research station at Kiel. This institution has six departments, and is well equipped and has just been reorganized. Eastern Germany offers opportunity for dairy research at the Institution of Research at Königsberg. It is at this institution that Dr. Grimmer edits a new and permanent dairy research journal, Milchwirtschaftliche Forschungen.

Government dairy research activities are also carried on at Halle by Professor Gutzeit; and at Weihensphan (near Münich) by Professor Fehr.

The above mentioned institutions have connections with the universities. Besides these there are other stations receiving private and

government support in Germany. These, however, do not have a direct connection with the universities.

Another step in the promotion of agricultural research has been the establishment of a research branch for agriculture at the University of Breslau. This project has included facilities for housing the new work which cannot be surpassed. The main building is as large and well constructed as buildings used for a similar purpose at universities in this country. The main new building at Breslau includes general offices, laboratories for the study of biochemistry, veterinary science, and agricultural sciences. Greenhouses, barns and other buildings have been provided for the work which will be conducted at this institution.

J. C. MARQUARDT,
New York Agricultural Experiment Station, Geneva, N. Y.

AMERICAN DAIRY SCIENCE ASSOCIATION MEMBERS FOR 1924

Below is published a list of the paid up membership of the American Dairy Science Association for 1924. Any reader of the JOURNAL, elegible to membership, whose name does not appear in this list should communicate at the earliest convenience with Secretary J. B. Fitch, Manhattan, Kansas. Prompt remittance of dues will insure the receipt of every number of the JOURNAL. It will also avoid unnecessary changes in committee appointments. Send your remittance today.—*Editor.*

ALTWEG, C. A.....Carnation Milk Company, Oconomowoc, Wis.
ASHBAUGH, V. J.....Adams, N. Y.
ANDERSON, E. O.....University of Nebraska, Lincoln, Nebr.
ATKESON, F. W.....Dairy Department, Moscow, Idaho
AREY, J. A.....Dairy Extension, State College Raleigh, North Carolina
ANTHONY, E. L.....Morgantown, West Virginia
ASHTROTH, FRANK B.....1502 Raymond Avenue, St. Paul, Minn.
ADAMS, DAVID P.....Department of Agriculture, Nashville, Tenn.
ANDERSON, G. C.....Dairy Department, Moscow, Idaho

BEAM, A. LELAND.....State College, Pa.
BECHDEL, S. I.....State College, Pa.
BORLAND, A. A.....Dairy Department, State College, Pa.
BRODY, SAMUEL.....Dairy Building, Columbia, Mo.
BRANDT, P. M.....Oregon Agricultural College, Corvallis, Ore.
BRIGHAM, ELBERT S.....St. Albans, Vt.
BESEMER, A. M.....California Central Creameries, Eureka, Cal.
BOUSKA, F. W.....1526 S. State Street, Chicago, Ill., Beatrice Creamery Company
BRANDT, CARL A.....745 North Avenue, Bridgeport, Conn.
BREW, JAMES D.....Dairy Department, Cornell University, Ithaca, N. Y.
BREED, ROBERT S.....Geneva, N. Y.
BROWN, R. W.....Agricultural College, Winnipeg, Canada
BUTTON, F. C.....Agricultural Experiment Station, Dairy Department, New Brunswick, New Jersey.
BECKER, RAYMOND B.....University Farm, St. Paul, Minn.
BECKMAN, H. C.....29 E. Madison Street, Chicago, Ill.
BRANNON, J. M.....Dairy Department, Urbana, Ill.
BUCHANAN, F. A.....Virginia Polytechnic Institute, Blacksburg, Va.

BURDICK, HOWLAND.....Rhode Island State College, Kingston, R. I.
BRYAN, K. V.....Dairy Department, West LaFayette, Ind.
BALTZER, A. C.....Dairy Department, East Lansing, Mich.
BENNETT, FREDERICK W.....Dairy Department, Georgia State College of Agriculture, Athens, Ga.
BAYER, A. H.....New Haven Dairy Company, New Haven, Conn.
BIGELOW, A. P.....Middlesex, Vt.
BOUDEWYNS, CELESTIN.....R. R. No. 1, Box 145, Westwood, N. J.
BOEHR, J. W.....Dairy Department, Stillwater, Okla.
BRITT, A.....City Consumers Company, Paducah, Ky.
BARBOUR, FORREST A.....Division of Animal Industry, Augusta, Me.
BRINTNALL, EARLE.....Starkville, Miss.
BIERMAN, HARLOW.....Experiment Station, College Park, Md.
BROWN, HAMLIN L.....Extension Dairyman, Gainsville, Fla.

CAINE, GEORGE B.....Logan, Utah
CALDWELL, R. E.....Blatchford Calf Meal Company, Waukegan, Ill.
CAVE, H. W.....Dairy Department, Manhattan, Kans.
CORBETT, L. S.....Orono, Me.
CUNNINGHAM, O. C.....New Mexico College of Agriculture, State College, New Mexico
CLUTTER, J. G.....College Station, Texas
COMBS, W. B.....Dairy Department, State College, Pa.
CLEVENGER, W. L.....State College, Raleigh, N. C.
COOK, ALFRED.....Plainsboro, N. J.
COOLEDGE, L. H.....East Lansing, Mich.
CAMPBELL, M. H.....Dairy Department, Urbana, Ill.
COLMAN, H. N.....Dairy Department, Corvallis, Ore.
COHEE, C. I.....1211 Arch Street, Philadelphia, Pa.
CAMBURN, O. M.....38 Academy Street, Arlington, Mass.
CUNNINGHAM, WALTER S.....Tucson, Ariz.
CUMMINGS, CHAS. M.....Peterboro, N. H.

DAVIS, H. P.....Dairy Department, Lincoln, Nebr.
DICE, J. R.....Agricultural College, North Dakota
DVORACHEK, H. E.....Fayetteville, Ark.
DAHLBERG, A. C.....Experiment Station, Geneva, N. Y.
DAHLBERG, A. O.....367 Grove Street, St. Paul, Minn.
DAHLE, CHESTER D.....University of Minnesota, St. Paul, Minn.
DRAIN, HARRY D.....Ohio State University, Columbus Ohio
DARNELL, A. L.....College Station, Texas
DOAN, F. J.....Dairy Department, College Park, Md.
DARGER, H. C.....Blue Valley Creamery Company, Chicago, Ill.
DUNNE, J. E.....Hazelwood Ice Cream Co., East 6th and Main Streets, Portland, Ore.
DE PEW, H. F.....State College, Durham, N. H.

ECKLES, C. H.....University Farm, St. Paul, Minn.
ELLENBERGER, H. B.....University of Vermont, Burlington, Vermont
EPPLE, W. F.....311 Sylvia Street, West LaFayette, Ind.
ENGBRETSON, A. E.....Experiment Station, Astoria, Ore.
ELLINGER, TAGE H.....Armour's Livestock Bureau, Chicago, Ill.

FRANDSEN, J. H.....Lincoln, Nebr.
FITCH, J. B.....Dairy Department, Manhattan, Kans.
FRASER, W. J.....Urbana, Ill.
FULLER, J. M.....New Hampshire College, Durham, N. H.
FISK, W. W.....Cornell University, Ithaca, N. Y.
FROST, M. D.....Agricultural Bacteriology Department, Madison, Wis.
FITTS, E. B.....Department of Agricultural Extension, State College, Pa.
FISHER, R. C.....Storrs, Conn.
FREVERT, G. E.....Newman, Cal.

GOODWIN, O. T.....Michigan Producers Dairy Company, Adrian, Mich.
GOSS, E. F.....Iowa State College, Ames, Ia.
GREGORY, H. W.....416 Harvey Avenue, LaFayette, Ind.
GUTHRIE, E. S.....Cornell University, Ithaca, N. Y.
GANNON, E. A.....Purdue University, LaFayette, Ind.
GEARHART, C. R.....Dairy Department, State College, Pa.
GAMBLE, J. A.....University of Maryland, College Park, Md.
GULLICKSON, T. W.....University Farm, St. Paul, Minn.
GREEN, SAM H.....216 Pine Street, c/o California Dairy Council, San Francisco, Cal.
GRAY, C. E.....California Central Creamery Company, San Francisco, Cal.
GARDNER, M. H.....Delavan, Wis.

HALE, JOSEPH C.....Akron Milk Company, Akron, Ohio
HIGGINS, L. A.....Starksville, Miss.
HAYDEN, G. C.....Experiment Station, Wooster, Ohio
HOOPER, J. J.....Kentucky Agricultural College, Lexington, Ky.
HOPPER, H. A.....Cornell University, Ithaca, N. Y.
HUFFMAN, CARL F.....Michigan Agricultural College, Dairy Department, East Lansing, Mich.
HAMMER, B. W.....Iowa State College, Ames, Ia.
HARDING, H. A.....Box 834, Detroit, Mich.
HASTINGS, E. G.....University of Wisconsin, Madison, Wis.
HUNZIKER, O. F.....Blue Valley Creamery, Chicago, Ill.
HARMON, E. M.....Columbia, Mo.
HOOVER, JESSIE M.....Dairy Division, Washington, D. C.
HUTTON, C. A.....Extension Division, Knoxville, Tenn.

HARRISON, HARTMAN, K.....Linden Avenue and Dolphin Street, Baltimore, Md.
HEINEMAN, H. E. O.....327 S. LaSalle St., Chicago, Ill.
HERZER, F. H.....Agricultural College, Mississippi
HOLDAWAY, C. W.....Blacksburg, Va.
HARVEY, SAMUEL H.....College Park, Md.
HANSEN, A. P.....Director State Experimental Creamery, Copenhagen, Denmark
HAMMOND, R. L.....3016 Broadway, Indianapolis, Ind.
HENING, J. C.....New York Experiment Station, Geneva, N. Y.
HASLERUD, E. J.....Dairy Department, Bozeman, Mont.
HINDLEY, R. W.....Horlick's Malted Milk Company, Racine, Wis.
HARRIS, ROY T.....Madison, Wis.
HOLLER, L. S.....Blue Valley Creamery Company, Cedar Rapids, Ia.
HILEMAN, J. L.....Iowa Dairy Company, Dubuque,-Ia.

JACKSON, H. C.....Cornell University, Ithaca, N. Y.
JEWELL, R. M.....618 High Street, Mt. Vernon, Ohio
JUDKINS, H. L.....Dairy Department, Amherst, Mass.
JONES, IDWAL R.....Dairy Division, University Farm, St. Paul, Minn.
JONES, H. M.....Dairy Specialist, Brookings, S. D.
JORDAN, H. C.....East Lansing, Mich.
JAMISON, NEAL C.....Extension Division, Corvallis, Ore.
KEENEY, M. H.....Cedar Grove, N. J.
KOCH, ARTHUR.....Minerva, Ohio
KOCH, IRVING O.....Moore-Ross Milk Company, Columbus, Ohio
KELLEY, FRANK W.....Gridley Dairy Company, Milwaukee, Wis.
KEITH, J. I.....Dairy Department, Moscow, Idaho

LARSON, C.....State College, Brookings, S. D.
LOCKWOOD, W. P. B.....58 Adella Avenue, West Newton, Mass.
LEHMKUHL, HENRY.....84 Exchange Street, Rochester, N. Y.
LA MASTER, J. P.....Dairy Division, Clemson College, S. C.
LITTLE, R. E.....139 N. Clark Street, Chicago, Ill.
LARSON, C. W.....Bureau of Dairying, Washington, D. C.
LATZER, R. L.....Hudson, Mich.
LIDE, B. M., JR.....2008 Pine Street, St. Louis, Mo.
LUSH, R. H.....Dairy Department, Manhattan, Kans.
LINDSLEY, H. A.....Box 1214, St. Petersburg, Fla.
LADD, JOHN E.....Animal Husbandry Department, Rhode Island State College, Kingston, R. I.
LUCAS, P. S.....Dairy Department, East Lansing, Mich.

MCDOWELL, J. C.....Bureau of Dairying, Washington, D. C.
MARTIN, G. L.....750 Fairview Avenue, St. Paul, Minn.
MEAD, S. W.....Agricultural Experiment Station, New Brunswick, N. J.

MONROE, C. F.....619 N. Beaver Street, Wooster, Ohio
MORRISON, F. B.....University of Wisconsin, Madison, Wis.
MACY, HAROLD.....University Farm, St. Paul, Minn.
MEISTER, ALBERT.....Capital Dairy, 315 Sixteenth Street, Sacramento, Cal.
MARTIN, WILLARD H.....State College, Pa.
MURRAY, JOSEPH.....Crescent Pure Milk Company, Winnipeg, Canada
MOON, M. P.....Dairy Department, Cornell University, Ithaca, N. Y.
MCCANDLISH, A. C. Claunch, Sorbie, Wigtownshire, Scotland
MANHART, V. O.....Dairy Department, West LaFayette, Ind.
MILLER, MILTON M.....1404 Raymond Avenue, St. Paul, Minn.
MCINERNEY, THOMAS J.....Dairy Department Cornell University, Ithaca, N. Y.
MOORE, J. S.....Agricultural College, Miss.
MCWHORTER, G. E.....Milledgeville, Ga.
MONRAD, K. J.....301 Mayer Building, Milwaukee, Wis.
MUDGE, COURTLAND S.....Davis, Cal.
MASON, R. H.....Dairy Department, Fayetteville, Ark.
MAUGHAN, M. O.....910 S. Michigan Avenue, Chicago, Ill.
MUNN, M. D.....910 S. Michigan Avenue, Chicago, Ill.
MIYOGISHIMA, MASU.....268 Hatagaya Yoyohata Mochi, The Fiyi Dairy, Tokio Japan
MOTOHASHI, HEIICHIRO.....Tottori Agricultural College, Tottori, Japan
MIYAWAKI, ATSUSHI.....Professor of Dairying, Imperial University, Sappora, Japan
MUSSER, KARL B.....Peterboro, N. H.
MARS, BRUCE S.....130 Russell Street, LaFayette, Ind.
MASUROVSKY, BENJAMIN.....Dairy Department, Lincoln, Nebr.

NEVENS, W. B.....810 Michigan Avenue, Urbana, Ill.
NAFIS, LOUIS.....544 Washington Boulevard, Chicago, Ill.
NELSON, D. HORACE.....Columbia, Mo.
NEWLANDER, J. A.....101 Brooks Avenue, Burlington, Vt.
NELSON, J. A.....Montana State College, Bozeman, Mont.
NORTON, H. W.....Lansing, Mich.
NYSTROM, A. B.....1819 G. Street, N. W., Washington, D. C.

OLSON, N. E.....Dairy Department, Manhattan, Kans.
OLSON, T. M.....Dairy Department, Brookings, S. D.

PARFITT, E. H.....Purdue University, LaFayette, Ind.
PONTIUS, JOHN A.....R. F. D. No. 2, Canton Ohio
PARKER, H. N.....Main and Orange Streets, English Building, Jacksonville, Fla.
POTTS, ROY C.....Department of Agriculture, Washington, D. C.
PEARSON, R. A.....Ames, Iowa
PAU, R. L.....4015 Commerce Street, Dallas, Tex.

PACE, D. J.....United States Experiment Station, Kodiak, Alaska
PHILLIPS, C. A.....Davis, Cal.
PREWITT, ED.....Dairy Department, Lexington, Ky.

RAGSDALE, A. C.....University of Missouri, Columbia, Mo.
REGAN, W. M.....Davis, Cal.
REID, WM. H. E.....Dairy Department, Columbia, Mo.
ROADHOUSE, C. L.....University of California, Davis, Cal.
ROSS, H. E.....Cornell University, Ithaca, N. Y.
RUEHE, H. A.....Dairy Department, Urbana, Ill.
REGAN, M. J.....Dairy Department, Columbia Mo.
ROBBERS, WALTER.....Dairy Department, New Brunswick, N. J.
ROGERS, L. A.....Dairy Division, Washington, D. C.
RENNER, K. M.....Dairy Department, Manhattan, Kans.
RUFFNER, R. H.....State College, Raleigh, N. C.
RIDER, WM.....Syracuse University, Syracuse, N. Y.
RIEL, E. G.....State Institute of Agriculture, Farmingdale, N. Y.

SMITH, R. W., JR.....Massachusetts Agricultural College, Amherst, Mass.
SAMMIS, J. L.....Madison, Wis.
SLATER, EDWARD K.....210 Sycamore Street, Milwaukee, Wis.
STOCKING, W. A.....Dairy Department, Cornell University, Ithaca, N. Y.
STOLTZ, R. B.....Dairy Department, Columbus, Ohio
SUPPLEE, GEORGE C.....Dry Milk Company Laboratory, Adams, N. Y.
SIDELMANN, I. O.....State College, Pa.
STUART, DUNCAN.....Bureau of Dairying, Washington, D. C.
SWETT, W. W.....Bureau of Dairying, Washington, D. C.
SHUTT, D. B.....Bacteriology Department, Agricultural College, Winnipeg, Manitoba, Canada
SPITZER, GEORGE.....1000 Seventh Street, West LaFayette, Ind.
SHEPHERD, J. B.....Bureau of Animal Industry, Ardmore, S. D.
SHELTON, FRED S.....Clemson, College, S. C.
SHERMAN, J. M.....Dairy Industry Building, Ithaca, N. Y.
SAVAGE, E. S.....Animal Husbandry Department, Cornell University, Ithaca, N. Y.
SCOTT, GEORGE W.....Dairy Instructor, Edmonton, Alberta, Canada

TURNER, CHAS. W.....Dairy Department, Columbia, Mo.
THOM, CHAS.....Bureau of Chemistry, Washington, D. C.
TROY, H. C.....Cornell University, Ithaca, N. Y.
THURSTON, L. M.....Dairy Department, University Farm, St. Paul, Minn.
TEGNER, CARL E.....Davis, Cal.

URNER, F. G.....Urner-Barry Company, 173 Chambers Street, New York City, N. Y.

VAN NORMAN, H. E.....910 S. Michigan Avenue, Chicago, Ill.
VAN LANGE, OSCAR.....340 S. Fremont Street, Los Angeles, Cal.

WHITE, G. C.....Storrs, Conn.
WILBUR, J. W.....Purdue University, LaFayette, Ind.
WILLIAMS, V. M.....Animal Industry Division, State College Station, Raleigh, N. C.
WING, H. H.....Cornell University, Ithaca, N. Y.
WYLIE, C. ELMER.....University of Tennessee, Knoxville, Tenn.
WINKJER, JOEL G.....Bureau of Dairying, Washington, D. C.
WELD, IVAN C.....Chestnut Farm Dairy, Washington, D. C.
WEAVER, EARL.....Ames, Iowa
WALTS, CHAS. C.....Dairy Department, Storrs, Conn.
WILLIAMS, G. A.....Purdue University, LaFayette, Ind.

American Dairy Science Association Meets at Milwaukee

CALENDAR OF EVENTS OF INTEREST TO MEMBERS AT THE NATIONAL DAIRY SHOW AT MILWAUKEE, SEPTEMBER 27 TO OCTOBER 5.

SATURDAY, SEPTEMBER 27

Students National Contest in Judging Dairy Cattle.

MONDAY, SEPTEMBER 29

Students National Contest in Judging Dairy Products.

9:00 a.m. General Session of the American Dairy Science Association.

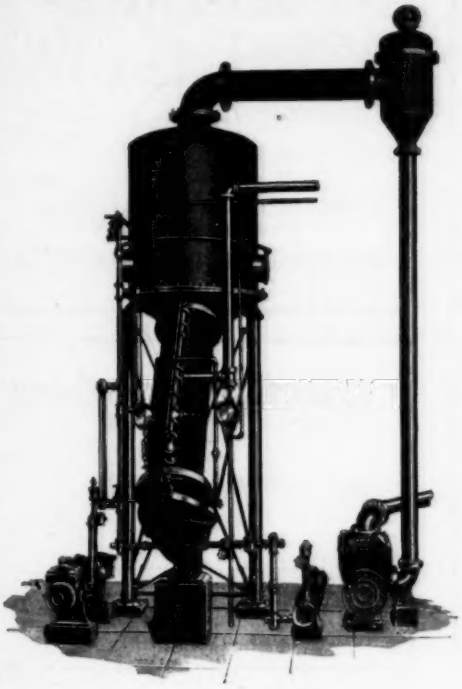
1:30 p.m. Sectional Meetings—Production, Manufacturing and Extension.

7:30 p.m. Sectional Meeting—Advanced Registry.

TUESDAY, SEPTEMBER 30

6:00 p.m. Banquet of American Dairy Science Association. Results of Contest.

MEETING PLACE, SPEAKERS AND HOTEL HEADQUARTERS TO BE ANNOUNCED LATER.



—For the Production of:

Evaporated Whole and Skim Milk

Sweetened Condensed Milk

Semi-Solid Buttermilk

BUFFLOVAK

Milk Evaporators

In addition to the concentration of skim milk and buttermilk, previous to drying, BUFFLOVAK Milk Evaporators afford a number of advantages in the production of evaporated whole and skim milk as well as sweetened condensed milk.

All of the original health-giving qualities and flavor are preserved in the finished product—brief contact with only moderately heated surfaces assures this.

And BUFFLOVAK Evaporators can be easily and thoroughly cleaned without entering the machine. Large hinged doors afford ready access to every part of the evaporator.

BUFFALO FOUNDRY & MACHINE CO.
1581 FILLMORE AVE., BUFFALO, N. Y.

NEW YORK: 17 Battery Place **CHICAGO: 1636 Monadnock Bldg.**

Your advertisement is being read in every State and in 25 Foreign Countries

GUERNSEYS

THE QUALITY-QUANTITY BREED

None are so familiar with the attributes of the Guernsey as the scientists of the dairy industry.

Agricultural Colleges have met the breed in its field of greatest success,—the cow testing association and the bull association.

Dairy extension men are invited to write us for detailed information regarding the breed.

THE AMERICAN GUERNSEY CATTLE CLUB
Box DS-103 PETERBORO, N. H.

MARSCHALL RENNET AND COLOR

STRONG PURE

DEPENDABLE

Made in the largest and best equipped Rennet factory in America!

THE MARSCHALL DAIRY LABORATORY
MADISON, WISCONSIN

Classified Advertising

$3.00 PER INCH

Journal of Dairy Science
Mount Royal and Guilford Avenues
BALTIMORE, MD.

HANSEN'S

There are many reasons why

HANSEN'S Rennet Extract

is superior:

Excels in strength and purity.
Contains no pepsin.
The most curdling power for your money.
Of the same curdling power always.
Superior in keeping quality.
Made from the finest rennets.

Six Out of Eight Highest Scores

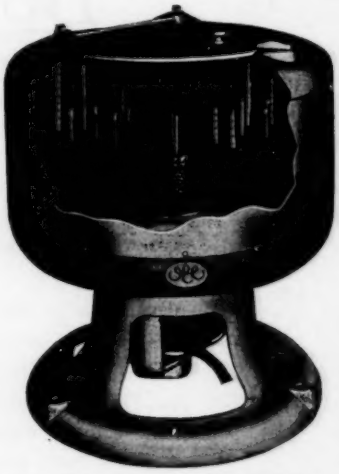
were made by users of **Hansen's Rennet**, at the 1923 National Dairy Show.

"Fifty Years of Knowing How Make Hansen's Rennet Better Now"

Chr. Hansen's Laboratory, Inc.
Little Falls, N. Y.

Western Branch MILWAUKEE, WIS.
Canadian Factory TORONTO, CAN.

BABCOCK TESTERS



See Jour. Dairy Sci., Jan. 21, 1921, page 32

INTERNATIONAL EQUIPMENT COMPANY
352 Western Ave. (Brighton)
BOSTON, MASS.

Your advertisement is being read in every State and in 25 Foreign Countries

Foresight

Imagination and foresight guard the future of industry by meeting and measuring conditions before they arise.

In the realm of science, dairy investigation, by preparing the way to a better understanding of milk values, has made valuable contributions to the advancement of human welfare, and a corresponding increased consumption of milk products.

Foresight also prompted the manufacture of

Wyandotte Dairyman's Cleaner and Cleanser

in anticipation of the need for a better and more efficient dairy cleaner to make practical the results obtained.

The best proof of the wisdom of this policy is found in the use of this cleaner in the Agricultural Colleges of the United States and Canada and in its increasing use in the dairies of the country. Order from your supply house. ***It cleans clean.***

Indian in circle

in every package.

Spend a Week in Dairyland

NATIONAL DAIRY EXPOSITION

MILWAUKEE, WIS.

September 27th to October 4th

The J. B. Ford Co., Sole Mnfrs., Wyandotte, Mich.

Your advertisement is being read in every State and in 25 Foreign Countries

DRAWINGS, ARTISTS' WORK AND ENGRAVINGS FOR MEDICAL AND SCIENTIFIC PUBLICATIONS

PUBLISHERS and authors working in the scientific field will be interested in our facilities for producing illustrations for such work. We have had years of experience in working in co-operation with some of the prominent authors and scientists of the Johns Hopkins University, which has developed in our organization an intimate knowledge of the requirements of this particular line of work. We offer you the services of the specialist in this line. Such are not available in every community, and as we are accustomed to working from written instructions, our clientele being largely at distant points, we subconsciously understand the illustrative point to emphasize, and have been very successful in satisfying our most critical patrons.

We will gladly make estimates, submit samples, and co-operate in every way possible in the production of such work.

BALTIMORE MARYLAND ENGRAVING COMPANY

BALTIMORE, MARYLAND

Your advertisement is being read in every State and in 25 Foreign Countries

BACILAC

DEHYDRATED MILK AGAR

BACTERIOLOGICAL CULTURE MEDIUM

BACILAC is dehydrated milk agar

—It eliminates the trouble of preparing milk agar
—It is compounded with tested ingredients
—It is uniform
—It is valuable for differential counts
—It is valuable for growing the Lacto bacillus group
—It produces higher colony counts from milk
—It has been tested in numerous laboratories
—It has been endorsed by authorized committees
—It has passed the experimental stage

AFTER YEARS OF INVESTIGATION AND COMPARISON WITH OTHER STANDARD MEDIA, BACILAC—DEHYDRATED MILK AGAR—IS NOW AVAILABLE FOR GENERAL USE.

MANUFACTURED BY

THE DRY MILK COMPANY

Research Department **15 PARK ROW, NEW YORK**

MILK, CREAM, BUTTER TESTING

QUICK **SIMPLIFIED** **ACCURATE**

MONEY SAVING

Test Tube

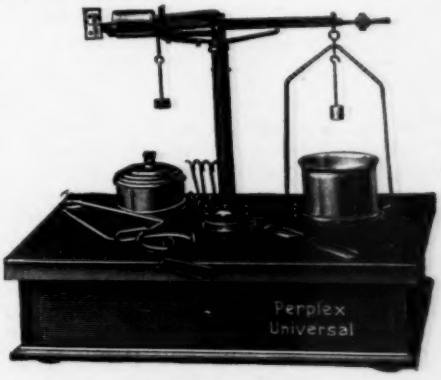
Other Specialties:

Test of Condensed-Dried-Milk, Cheese

Ice Cream by the New Troy-Fucoma Method

Methylene Blue Test
Catalase Test

Complete line of most accurate Thermometers, Lactometers, etc.



Butter Moisture Scale

FUCOMA COMPANY, Inc.

154 NASSAU STREET NEW YORK

Laboratory Supplies For Dairies, Creameries, Butter and Cheese Factories. Write For Our Illustrated Circulars

Your advertisement is being read in every State and in 25 Foreign Countries

Military Meat and Dairy Hygiene

A comprehensive manual compiled by Captain H. S. Eakins, Veterinary Corps, Medical Department, U. S. Army under the direction of the Surgeon General.

Prepared especially as a manual of standard requirements and methods for inspection of meats and dairy products for use by the United States Army. The book will be of decided value to everyone interested in the production, handling, or sale of meat and dairy products.

Production, grades, methods of grading, preparation and use, packing, cold storage, shipping of meat and dairy products are discussed.

There are chapters on sanitary examinations, inspection of dairy farms and plants, ice manufacture, requirements for certified milk, eggs, fish and other sea foods.

Many illustrations, explanations, and detailed instructions help to make the book clear and explicit. A complete index is a feature.

Price, $6.50, postpaid

ORDER DIRECTLY FROM THE PUBLISHERS

WILLIAMS & WILKINS COMPANY

BALTIMORE, MARYLAND

Short Histories of Many Inventions and Discoveries in the Field of Science

POPULAR RESEARCH NARRATIVES

Compiled by ALFRED D. FLINN of Engineering Foundation

A book of fifty little five-minute stories that reveal the true origin of many scientific discoveries and inventions. Written by "men who know."

The series was originally issued in pamphlet form for semi-monthly distribution to a small group. So many were the demands for a more permanent form that the first fifty narratives (the series is still being continued) have been issued in book form; to be followed by another fifty in book form when available.

Gore once said, "the origin of many important discoveries lies buried in the obscurity of past ages." That statement was made before Research Narratives was issued, for in it many of these heretofore unknown origins have been revealed.

In this book is a fund of material for the teacher, the after dinner speaker, the lecturer, the student, the parent. You will want several copies at the price—50 cents.

ORDER ANY QUANTITY AT 50 CENTS PER COPY

FROM THE

ENGINEERING FOUNDATION

29 W. 39th St., New York City

OR FROM THE PUBLISHER

WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, MARYLAND

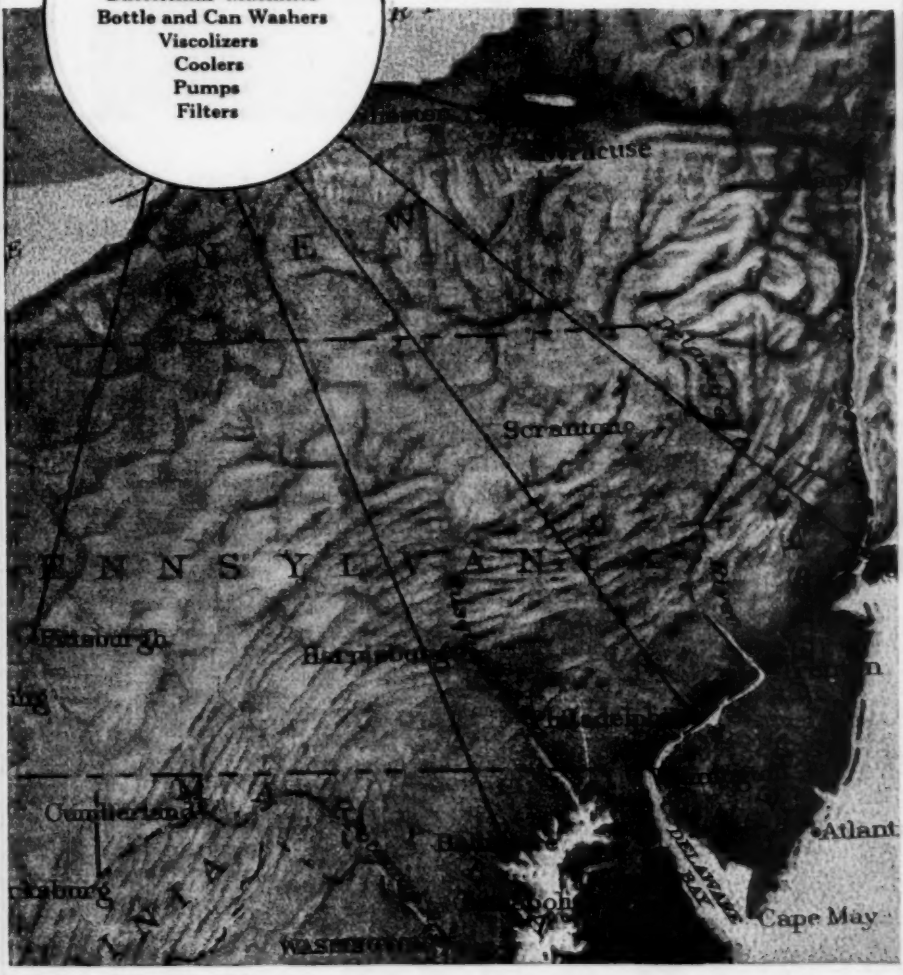
Your advertisement is being read in every State and in 25 Foreign Countries

THE BESTOV CALENDAR

Complete Equipment to Handle Milk — from Cow to Consumer

AT YOUR SERVICE!

Churns
Freezers
Pasteurizers
Buttermilk Machines
Bottle and Can Washers
Viscolizers
Coolers
Pumps
Filters

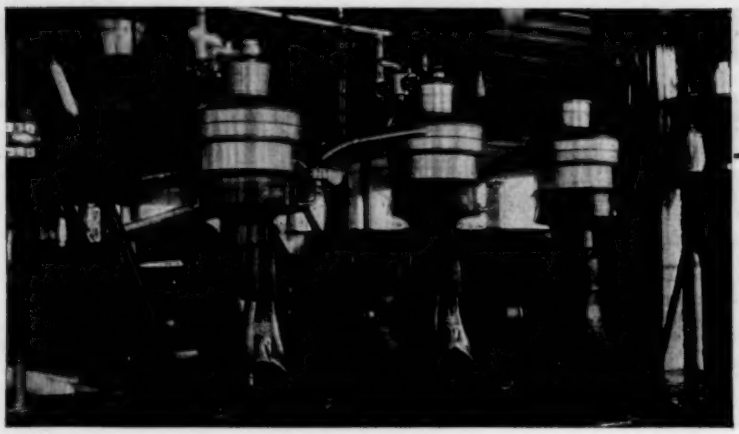


FIVE BIG STORES →

PHILADELPHIA
BALTIMORE
PITTSBURGH
SYRACUSE
NEW YORK CITY

Cherry-Bassett-Winner Company

Your advertisement is being read in every State and in 25 Foreign Countries



"The average loss of butter-fat has not exceeded one one-hundredth of one per cent"

—writes the Ovid Creamery Company, Ovid, Mich. "Our De Lavals have skimmed better than an average of 50,000 pounds of milk a day, and needless to say we are well pleased with them."

The really important point about De Laval Separators is that they do this kind of work day after day, and year after year, with the milk or whey in any reasonable condition and at any reasonable temperature.

In the spring when cows are fresh, and in plants where it is economical to skim at Pasteurizing temperature, it is comparatively easy for almost any reasonably good separator to skim clean. But in the fall and winter, when separation is more difficult, and in plants where the cost of heating and cooling milk or whey is an unnecessary expense, the De Laval also skims clean. In addition, it produces under any of these conditions cream of the desired density, free from taints or odors, and with the butter-fat globules in a natural condition.

These are the most important points to consider in choosing a separator and they apply almost equally to the creamery, cheese factory or milk plant. Consider their importance in your particular business, and you will understand why separator users who are producing quality products and earning maximum profits invariably choose the De Laval to get them the most butter-fat, in the best condition, at the least cost.

The De Laval Separator Company

NEW YORK
165 Broadway

CHICAGO
29 East Madison Street

SAN FRANCISCO
61 Beale Street

Sooner or later you will use a

De Laval

Your advertisement is being read in every State and in 25 Foreign Countries

Your Trouble Eliminated At Last

As the result of many years persistent experimenting we are now in a position to offer to the Creamery Trade

Wagner's Precision Test Bottles

WITH PERMANENT BLACK ETCHED GRADUATION

which we guarantee to withstand acid or the strongest alkali solution commonly used in cleaning test bottles.

We guarantee this graduation never to decolorize. It enables you to read your test correctly at all times; saves your eyesight; you need not be troubled any longer with disappearing invisible graduations, a most important feature which is highly appreciated by all users. Some of the testimonials received, read:

"Your Test Bottles With Permanent Black Etched Graduation Are Worth Their Weight in Gold."

Owing to the unusually strong demand, our manufacturing resources are already taxed to the limit. We, therefore, ask you to place your orders in time.

Ask for our illustrated catalog for testing milk and its products.

ESTABLISHED 1867

Awards

Panama International Exposition

Louisiana Purchase Exposition

THE WAGNER GLASS WORKS, 695-697 E. 132nd St., New York, N.Y., U.S.A.

Inventors and Manufacturers of Scientific Glassware and Apparatus for Testing Milk and its Products

DIFCO

BACTERIOLOGICAL REAGENTS "DIFCO-STANDARDIZED"

Recommended for use in the Bacteriological Analysis of Dairy Products

	Bacto-Peptone	
Bacto-Beef	Bacto-Agar	Bacto-Dextrose
Bacto-Beef Extract	Bacto-Gelatin	Bacto-Lactose

DEHYDRATED CULTURE MEDIA "DIFCO-STANDARDIZED"

Bacto-Nutrient Broth	Bacto-Dextrose Broth
Bacto-Nutrient Gelatine	Bacto-Lactose Broth
Bacto-Nutrient Agar 1%	Bacto-Whey Agar

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS

In the research and development of Bacto-Peptone and Dehydrated Culture Media

DIGESTIVE FERMENTS COMPANY

DETROIT, MICHIGAN, U. S. A.

THE STANDARD FOR NEARLY FORTY YEARS

TRADE MARK

Paterson Vegetable Parchment

"Nearest to Perfection"

Nearly forty years ago we were making Vegetable Parchment.

One does not specialize for nearly forty years without coming very close to Perfection.

The Paterson Parchment Paper Co.

Passaic, New Jersey